8-2016

Love That Dirty Water : A Preliminary Investigation of Microbial Diversity in On-Street Hot Dog Water

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LOVE THAT DIRTY WATER: A PRELIMINARY INVESTIGATION OF MICROBIAL DIVERSITY IN ON-STREET HOT DOG WATER

by

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A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science

August 2016

College of Science and Mathematics

Department of Biology

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Abstract

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Standard microbiological methods were utilized to assess the microbial diversity and abundance in the hot dog water found in mobile food carts. Since we could not directly assay the water, we devised a method of washing hot dogs with sterile water to indirectly assess the presence of microorganisms both growing in the water and on the hot dogs. Plating on LB-agar and LB-agar supplemented with antibiotics was used to isolate microbes from hot dog water from randomly selected vendors in twenty-six Manhattan neighborhoods and the Bronx. In addition, five major hot dog brands were similarly tested as store-bought controls. As expected, on-street hot dog water contained culturable microbes, ranging from 0 to more than 1.25×10^5 Colony Forming Units (CFU's) per hot dog (X = 8,000 CFU's/hot dog). Control hot dogs had considerably fewer culturable microorganisms (from 0 to to 2.5×10^3 CFU's per hot (X = 1,100 CFU's/hot dog). We have also discovered antibiotic resistant bacteria at 5 of our 26 sites (20%), including Ampicillin, Chloramphenicol, Kanamycin and Tetracycline resistance. In addition, our data suggests a possible correlation between CFU's and serving temperature of hot dogs. Those served at temperatures of 145 F or greater had a mean of 33.9 CFU's/hot dog, whereas those below 145 F had a mean of 9,897 CFU's/hot dog. PCR-based amplification using the 16S locus for DNA barcoding was utilized to identify isolated colonies. We identified 22 different microbial species in on-street hot dogs, and 10 different species in store-bought (control) hot dogs. In summary, the hot dog water microbiome is complex and shows greater diversity than that associated with control hot dogs.

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Introduction

The Great American Hot Dog: A Brief History

The most important fact to know about the great American hot dog is, well, it's not American. Hot dogs were first made in Europe, but quickly became popular in the United States since these sandwiches were both inexpensive and convenient. As such, they have become the staple of American baseball, picnics, barbeques, and are sold on street corners in most major American cities.

The American hot dog was developed from the German Frankfurter and Austrian Weiner. Johann Georg Lahner created both sausages, and named them after the cities in which they were first made, Frankfurt, Germany and Vienna, Austria (Glass, 2016). Lahner was a butcher in Frankfurt, Germany in the late 1700s, where he created the Frankfurter. He later moved to Vienna, Austria, where he combined pork and beef to create the first white sausage, more commonly known as the hot dog (Glass, 2016).

White sausages were first consumed in the US in the early 1800s, but people did not eat them with bread until the 1860s. The sausage making technique was initially viewed as a way to use the undesirable meats of cattle and swine (Smith, 2006). Fats, genitalia, and other less-edible parts of cows and pigs were ground or chopped, and blended with salts, spices, and herbs. Intestinal casings were filled with this meat mixture before being sliced into smaller hot dogs (Smith, 2006). People usually ate hot dogs at fairs and during other outdoor festivities. In 1867, Charles Feltman was the first vendor to sell hot dogs on the streets of Coney Island (Smith, 2006). He started by selling them off of a pushcart near the entrance of an amusement park. His business became so lucrative that he was able to open a restaurant to sell his hot dogs (Smith, 2006). One of

the waiters he employed was a Polish immigrant named Nathan Handwerker. In 1916 Handwerker decided that he would leave his job at Feltman's restaurant and open one of his own. Handwerker's hot dog business, named Nathan's Famous, quickly flourished, and continues to sell hot dogs along the East Coast (Smith, 2006) and sponsors an annual hot dog eating contest on the 4th of July in Coney Island (Nathan's Famous International Hot Dog Eating Contest, n.d.).

By the early twentieth century, hot dogs were sold throughout the United States. Hot dogs are inexpensive to produce, and can be sold for a cheaper price than most other sandwiches. Consequently, vendors began to sell hot dogs at baseball games for their convenience and price (Smith, 2006). Vendors began to build their own empires around the white sausages. Oscar Mayer, for example, was a German immigrant who began making white sausages in a northern Chicago butchery (Smith, 2006). His hot dog business grew into what is now known as the Oscar Mayer brand, which now sells many kinds of meat, including the classic hot dog (Smith, 2006). Increased hot dog consumption sparked the development of various fast food businesses. For instance, two brothers, Maurice and Richard McDonald, started a hot dog business near the Santa Anita racetrack in Los Angeles County, CA. Their business later became McDonald's after hamburgers were added to the menu, but much of their early success originated from their hot dog business (Smith, 2006). Another fast food business, also based in Los Angeles, became a world-famous restaurant for selling hot dogs. John Galardi opened Wienerschnitzel in 1961, which later became one of the most popular hot dog restaurants in the US (Smith, 2006).

How do you make a hot dog?

To produce hot dogs, manufacturers obtain beef, pork and/or chicken trimmings from an outside supplier. The trimmings are inspected and then enter the sausage making process. If the meat passes all inspections, the trimmings are first ground to a consistency similar to that of hamburger meat (Lutz, 2013). Chicken trimmings, food starch, salt, and various spices are then added. Then, water and corn syrup are incorporated into this mixture. The batter is then mashed to a fine grain, and air is removed from the middle of the mixture to remove air bubbles (Lutz, 2013). The puree is then stuffed into a cellulose casing and twisted into five and a quarter inch sausages. The sausages are first placed through a liquid and smoke shower and then through an oven where they cook zones (Lutz, 2013). To prepare for packaging, the hot dogs are placed in a salt water shower. The final step is the removal of the cellulose casing. Lastly, hot dogs undergo another inspection before being packaged. During packaging, the hot dogs are placed in a machine where they are divided up into groups and sealed in between plastic. The processed hot dogs are then shipped refrigerated to vendors.

Government Oversight of Food Safety

1. United States Department of Agriculture (USDA)

As the hot dog industry continued to expand, it became necessary to ensure these sausages were being produced in an ethical and sanitary manner. This prevents businesses from taking short cuts at the expense of human health and animal welfare. More specifically, laws were written to minimize bacterial contaminations and stop the unethical treatment of animals used for meat (HHS.gov, 2016). Bacterial infections are of particular concern because they can quickly spread and cause severe illnesses,

depending on the type of bacteria (HHS.gov, 2016). The US Department of Agriculture (USDA) is a branch in the government that protects and enforces medical, public and social health service laws (HHS.gov, 2016). In the hot dog business, the USDA protects consumers by enforcing laws on both the hot dog manufacturers and the mobile cart vendors that sell the sausages on the streets (HHS.gov, 2016).

The USDA includes hot dogs in the Federal Meat Inspection Act. This law focuses on animal welfare and meat quality (Title-21_603, 2010). Under this act, animals must be slaughtered humanely such that the animal feels minimal pain (Title-21_603, 2010). Any animal exhibiting symptoms of injury or illness are separated and slaughtered, and this meat is not consumed to prevent bacterial infection (Title-21_603, 2010). Once humanely slaughtered, animals undergo a post-mortem inspection, where meat that is unsafe for consumption is destroyed (Title-21_604, 2010). All meat that can be used for human consumption is shipped to manufacturing businesses, where it is inspected again (Title-21_605, 2010). Before being shipped, every meat-containing package must be labeled with what type of animal the package contains, along with which parts are included. It must also include the inspection status of said meat and the size of container (Title-21_607, 2010). The inspections are not limited to the meat itself, the USDA also regularly inspects the slaughtering and packaging establishments. Sanitation inspectors are called upon to verify and conduct regular checks for cleanliness and hygiene (Title-21_608, 2010). These sanitary and meat examinations can be performed at anytime of the day by the inspector; thus, the manufacturer must be prepared for such checks (Title-21_609, 2010). If any establishment is to be inspected, the government asks that said company continue with regular procedures and to keep a

written record of all regular practices (Title-21_613, 2010). These inspections can lead to a variety of outcomes, including fines or even imprisonment if standards are not met (Title-21_676, 2010). Such scrutiny is needed to ensure that meat products are of high quality and will not cause illnesses in consumers.

2. Department of Health for New York City (NYC Health)

In addition to the federal government, state and local agencies also have oversight and legislate food safety laws for meat vendors. Since our study was limited to New York City it seemed prudent to examine the local health codes relating to safety of mobile hot dog vendors.

The NYC Department of Health categorizes Mobile Food Vendors into five groups (Class A, B, C, D, and E) based on the type of food being sold and how it is prepared (see Table 1 below). More specifically, vendors are categorized based on whether their foods are processed, packaged, or hazardous (Article 81, n.d.). The NYC Department of Health places hot dog vendors under class D even though hot dogs are potentially hazardous. However, Chapter 6 of the Title 24 further explains that frankfurters are classified as potentially hazardous. The foods are classified in these five groups because of what vendors can sell in one cart. Class D allows for vendors to sell breakfast foods and beverages as well as some lunch and snack foods like boiled hot dogs (Article 81, n.d.). In this class the vendors are able to sell hot dogs, but they must be at a specific temperature. In the state of New York vendors must cook and keep tenderized and injected meats (like hot dogs) at a temperature of 155° F or 68° C (Article 81, n.d.).

Vendor Classification Divisions

Class A:	Vendors that serve raw potentially hazardous foods.
Class B:	Vendors that serve potentially hazardous foods that are manufactured or pre-cooked.
Class C:	Vendors that serve only intact, prepackaged potentially hazardous foods requiring temperature control for safety.
Class D:	Vendors that serve only non-potentially hazardous packaged or unpackaged foods that do not require temperature control for safety.
Class E:	Vendors that serve on green carts that sell only non-potentially hazardous unprocessed whole fruits and vegetables.

Table 1. Vendor Classification Divisions (Department of Health and Mental Hygiene Commissioner of Health and Mental Hygiene: Notice of Adoption of the Repeal and Reissuance of Chapter 6 of Title 24 of the Rules of the City of New York, 2012).

The hot dog vendors must also keep their carts up to the Department of Health standards and the carts must also pass health inspections. The state's checklist focuses on sanitation, and how the cart should be designed to accommodate maximum cleanliness and sanitary equipment. This emphasis on sanitation is designed to keep the outside environment (dirt and dust) out of the food being sold by the vendors (Article 81, n.d.). All surfaces and equipment that might or might not be in contact with food are all closely regulated. The government requires vendors to use hard, smooth and nonporous equipment. In addition, equipment that is used for holding potentially hazardous foods like hot dogs are to be kept in water bath which is at 135 degrees Fahrenheit or 57 degrees Celsius. In addition, once the day is over the utensils and the carts are to be cleaned with chemicals registered as anti-microbial pesticides with the US Environmental Protection Agency. Other

conditions regulated by the government include lighting, ventilation, plumbing, waste, and storage. Table 2 depicts requirements for each class of mobile food vendors.

Supply and Equipment Requirements

	Class A:	Class B:	Class C:	Class D:	Class E:
Potable water	YES	YES	NO	YES[1]	NO
Culinary sink	YES	YES	NO	NO	NO
Hand wash sink	YES	YES	NO	NO	NO
Waste water tank	YES	YES	YES[2]	YES[2]	NO
Overhead structure	YES	YES	YES	YES	YES
Ventilation	YES	YES	NO	YES	NO
Cold holding	YES	YES	YES	YES[2]	NO
Hot holding	YES	YES	YES	YES[2]	NO
Thermometers	YES	YES	YES	YES[2]	NO

[1] If generating liquid waste, like hot dog water.
[2] Hot and cold handling equipment required for potentially hazardous food, like hot dogs.

Table 2. Supply and Equipment Requirements (Department of Health and Mental Hygiene Commissioner of Health and Mental Hygiene: Notice of Adoption of the Repeal and Reissuance of Chapter 6 of Title 24 of the Rules of the City of New York, 2012).

Food Microbes

Based on the food safety regulation implemented by both the United States Government and the New York Health Department the hot dog vendors and hot dog manufacturers don't have much maneuverability. However, such regulations are prudent since microbe scares in foods are always occurring. In July of 2015, nineteen people became ill from *Listeria* in Connecticut, Missouri, New York, Indiana, Massachusetts, Michigan, New Jersey and Pennsylvania (Multistate Outbreak of Listeriosis Linked to

Packaged Salads Produced, 2016). *Listeria* is a genus of bacteria that contains mostly non-pathogenic species; however, it also contains two pathogenic forms *Listeria monocytogenes* and *Listeria ivanovii* (Taxonomy Browser, NCBI). *L. ivanovii* is a bacterium commonly found on plants and animal in stomachs, manly in ruminant animals. *L. ivanovii* is a gram-positive non-spore forming bacillus, and its pathogenicity is rarely seen in humans (Guillet *et al.*, 2010). *L. monocytogenes* is also found in plants; however, it is very commonly found in raw foods like meats and salads. Its extreme pathogenicity in humans is due to how difficult it is to kill these bacteria. *L. monocytogenes* is resistant to freezing, dry or hot temperatures, even though it lacks the ability to form spores (Azizoglu *et al.*, 2009). Its ability to reproduce in the freezer, is what caused the infection of the nineteen people. Once the Center for Disease Control (CDC) found out that these people all had the same illness they decided to conduct an investigation. The investigation began in September of 2015 and was finally resolved in April of 2016. The CDC found that all of these people ate a similar brand of mixed salads. The Dole Fresh Vegetable Inc. had to recall all the mixed salads that were packed around the same period of time as that was the product that made people sick. The CDC reports that of the nineteen people that were infected, one person died as a result of the infection (Multistate Outbreak of Listeriosis Linked to Packaged Salads Produced, 2016). *L. monocytogenes* causes Listeriosis - a disease that has headaches, confusion and stiff neck as its symptoms for non-pregnant women and men. While pregnant women can have similar symptoms, they may also suffer from miscarriage, stillbirth, and life threatening outcomes for their newborn (Definition & Symptoms: Listeria (Listeriosis, 2014). The government used the Food and Drug Administration (FDA) to further

investigate how exactly the *L. monocytogenes* got into the salad. This led to the temporary closing of one of Dole's salad facilities in (list city, state here). It is believed that Dole was aware of the possible *L. monocytogene* bacterial contamination for over a year (before the closing of the plant) (Beach, 2016).

L. monocytogenes is one of the bacteria on the FDA's list of potentially hazardous food microbes (Food Code, page 624, 2013). In some cases the FDA requires manufacturers to reduce the amount of oxygen in packages so that both *Clostridium botulinum* or *Listeria monocytogenes* are reduced or eliminated. Other bacteria on the FDA list are *Escherichia coli, Salmonella spp., Listeria innocua, Streptococcus faecalis, Staphylococcus aureus, Vibrio parahaemolyticus, Campylobacter spp., Brucella spp.,* pseudomonads (or aerobic yeast) and molds (Food Code, page 624, 2013).

Oxygen reduction is one of the many ways manufacturers are able to eliminate the growth of bacteria in their food products (Food Code, page 18, 2013). Oxygen reduction is a process of detecting what amount of oxygen a specific bacteria requires to stay alive, doing the opposite will result in the death of some bacteria (Black, 2004). A few other different techniques are use to eliminate different bacteria; for example, salad manufacturers can't use freezing as a sterilization technique to kill *L. monocytogenes* because they are able to live through extremely cold temperatures. Thus, the salad factories must use other techniques to stun the growth of this pathogenic bacterium. Hot dog manufacturers use oxygen reduction as one of the techniques to kill a variety of bacteria. However, *L. monocytogenes* is still able to survive under these anaerobic conditions. This ability for the bacterium to survive in these conditions causes the USDA to sometimes have manufacturers recall their food products. These inspections and recall

are done by a sector in the USDA called the US Department of Agriculture's Food Safety and Inspection Service (USDA/FSIS). The FSIS division does inspections of various food and this may lead to an identification of a potential pathogen by detecting its genome in the food source. In 2015 the USDA/FSIS recalled around thirty meat and meat-related food shipments, and from January to May of 2016 they recalled another thirty meat shipments (FSIS, n.d.). To illustrate the extent of the problem, Morgan Wallace's lab was able to demonstrate the presence of *L. monocytogenes* in 550 of 33,000 hot dog packages tested, a rate of 1.66% (Wallace, 2002). Wallace and his colleagues used the USDA/FSIS protocols to isolate *L monocytogenes*, the causative agent of Listeriosis, from packaged hot dogs. His study illustrates just how intractable this problem of food safety in processed products can be. Even with rigorous government oversight, regulation and inspection it is virtually impossible to eliminate the presence of potentially pathogenic bacteria from processed foods.

Molecular Identification of Bacteria using the 16S rDNA Locus

We live in a microbial world and current estimates, using scaling laws to predict microbial diversity, suggest that our planet is home to upwards of 1 trillion (10^{12}) bacterial species (Locey & Lennon, 2016). Bacteria are found in every part of our planet and they are responsible for myriad chemical and biogeochemical processes that have transformed our environment (Sun et al., 2015; Schloss et al., 2004). Such staggering abundance make the identification of bacteria a daunting process. Traditionally, microbiologist have relied upon cell morphology, cell staining, growth on various types of media, colony phenotypes, as well as biochemical and physiological assays for

classification. Presently, bacterial identification has been revolutionized by the use of modern molecular techniques, in particular rapid DNA sequence analysis of genetic barcodes. Although there is still debate over which DNA barcodes are most universally applicable, for prokaryotic identification the 16S locus reigns supreme.

The 16S ribosomal gene (rDNA) is *ca.* 1500 bp long and ubiquitous in all prokaryotes. This gene is transcribed to produce the 16S rRNA that is essential for the structure and function of the small ribosomal subunit in prokaryotic translation. This approach was pioneered in the 1980's by Carl Woese and others (Clarridge, 2004), where they found that the 16S rRNA gene was ideal at distinguishing different organisms. This locus has the optimal mix of conserved and variable elements that permitted the anchoring of DNA primers in the former, and sequence analysis through the latter regions to provide a unique identification (Janda, 2007). In addition, the success of this region can be seen in the abundance of 16S rDNA sequences in genetic databases which facilitates identification of unknowns. All in all, this makes the 16S rRNA sequence an excellent target for phylogenetic analysis.

The function of 16S rRNA is directly correlated with its shape and topology of this molecule. In *E. coli,* this rRNA molecule contains a little over 1500 bp with about 320 U's, 390 A's, 360 C's and 490 G's; its C-G count is high with an average of about seventy percent. The 16S gene contains nine hypervariable regions which alternate with conserved sections. The variable fragments are of all different sizes, the smallest containing just 31 nt while the largest comprises 106 nt. These conserved regions allow for primer binding and the variable regions allow for phylogenetic sorting (Yarza, 2014).

The hypervariable regions are species specific meaning they vary between different taxonomical species but the gene is the same amongst the same species. The variable regions are from nucleotides 69-99 (V1), 137-242 (V2), 433-497 (V3), 576-682 (V4), 822-879 (V5), 986-1043 (V6), 1117-1173 (V7), 1243-1294 (V8) and 1435-1465 (V9). While the conserved regions are from nucleotides 1-68 (C1), 100-136 (C2), 243-432 (C3), 498-575 (C4), 683-821 (C5), 880-985 (C6), 1044-1116 (C7), 1174-1242 (C8) and 1295-1434 (C9). When this gene is transcribed into a rRNA molecule it undergoes spontaneous folding that results in a unique secondary structure. The topology, size, and folding of the loops can also be helpful to scientists in understanding any changes to the rRNA (Patel, 2001).

Generation of amplicons from the prokaryotic 16S locus, followed by DNA sequence analysis of variable regions, has become the gold standard for bacterial identification. Although it is not without problems. For example, in rare cases some bacteria have been found to contain multiple and varied copies of the 16S gene. Others, although demonstrated to have different 16S genotypes are phenotypically identical in all other aspects. In an attempt to standardize this analysis, Yarza (2014) and his colleagues have catalogued the percentages at which each regions within this locus could be considered as the same genus, family, order, class and phylum. Others have suggested that using this approach one should consider a homology of 99.5% as a cutoff for identification at the species level (Janda & Abbott, 2007).

Research Aims:

Specifically, this research will seek to answer two questions:

1. By examining culturable microbes, to quantitatively assess the microbial population in on-street hot dog water when compared to control (store-bought) hot dogs.
2. Utilize the method of colony PCR to qualitatively identify, preferably at the species level, the bacteria found in both field-collected and control hot dogs.

Materials and Methods

A. Field Collections

Hot dogs were collected from March through August 2015. Twenty-five neighborhoods were targeted throughout Manhattan (see Table 3 and Figure 1). Vendors in each neighborhood were randomly selected and we purchased three hot dogs from each vendor. We requested just the three hotdogs (no bun, no condiments). Vendors typically placed the hot dogs in aluminum foil. We immediately recorded temperatures using an infrared thermometer (Nubee, MNUB8380, www.nubeestore.com). This infrared thermometer was not calibrated during any of the times uses in this experiment. Samples were quickly transferred into a sterile 36 oz. Whirl-Pak® Bag (Part# B01449WA, Nasco Science https://www.enasco.com/whirlpak/). The bag was sealed, labelled and stored on ice for transport back to the laboratory. All precautions were taken to avoid contamination of hot dogs after purchase from vendors. All of the field collections took place in Manhattan except for one sample from Morris Park (Bronx, NY).

B. Processing of Field Samples.

All field collected hot dogs were returned to the laboratory on ice and processed as soon as possible. All work was performed in a laminar flow hood (Labconco Corporation, Kansas City, MO; 3980403 and 3612500) that had been wiped down with 70% ethanol prior to processing. Twenty-five mL of sterile water was pipetted into each Whirl-Pak bag, the bag was resealed, and the sample vigorously shaken for 60 seconds. Afterwards the pooled liquid was removed with a sterile 25-mL pipette and transferred to a 50-mL sterile centrifuge tube (BioExpress GeneMate, Kaysville, UT; C-3394-3 50 mL tubes). An aliquot (1000 µl) was removed from each washing and held on ice for subsequent bacteriological plating. The remaining liquid (*ca.* 24 mL) from each washing was filtered through a 0.45 µM filter (Nalgene 115 mL, cellulose nitrate, Part No. 121-0045, Thermo Scientific) using vacuum and the filters stored at -80C until extraction. Barrier tips were used for micropipetting whenever possible.

C. Detection of Culturable Bacteria.

To detect the presence of culturable bacteria, 100 µL of each washing was spread onto a standard petri dish (94 x 16 mm) containing LB agar (Luria-Bertani; 37 grams per liter) using standard methods (Maniatis ref). In addition, 100 µl aliquots of each washing were also spread on plates containing LB-AMP (Ampicillin, Na salt, 50 µg/mL), LB-KAN (Kanamycin Sulfate, 50 µg/mL), LB-TET (Tetracycline, 12.5 µg/mL), and LB-CHL (Chloramphenicol, 30 µg/mL). All plates were incubated at 37C for 24 to 48 hr and colonies enumerated and photographed.

Individual colonies were isolated and re-plated onto a fresh LB agar plate or LB agar plate containing the appropriate antibiotic. These colonies were then picked with a sterile loop to extract total DNA from the colony for DNA barcoding using the 16S locus as detailed below.

D. Colony DNA Isolation.

Total DNA was isolated from individual colonies using the InstaGene Matrix reagent (Bio-Rad Laboratories, Hercules, CA; Catalog #732-6030) following manufacturers directions. Briefly, a single colony was picked with a sterile loop and added to a 1.7 mL Eppendorf tube containing 1 mL of sterile water. The colony was resuspended by pipetting or brief vortexing, pelleted in a microfuge (1 min @ 12,000 rpm), and the supernatant removed. Two-hundred µL of InstaGene matrix was added to the pellet and incubated for 25 minutes at 56C. Samples were then vortexed at high speed for 10 seconds, placed in a boiling water bath for 8 minutes, vortexed for another 10 seconds and then spun in a microfuge (12,000 rpm) for 2.5 minutes.. The supernatant is removed, being careful to not to disturb the pellet, and transferred to a new Eppendorf tube. All extracted colony DNA samples were stored at -20C until needed for PCR amplification.

E. PCR Amplification.

Polymerase Chain Reaction (PCR) was used to amplify DNA for molecular identification of bacterial species using the 16S rDNA locus (Woo,

2008). The primers used for amplification of this locus are listed in Table 6. All primers were synthesized by Integrated DNA Technologies (www.idtdna.com) and resuspended to a 100 μM stock in sterile deionized water and stored at -20C. Working concentrations of 10 μM were produced by dilution of the 100 μM stock. PCR reaction volumes were standardized at 20 μL and we utilized Choice Taq Master Mix (Denville Scientific, Denville, NJ; http://www.denvillescientific.com) for all amplifications. Reactions typically were produced by making a master mix (which contained all components except for template DNA) and then dispensed 19 μL each into 200-μL thin-walled Eppendorf tubes (BioExpress GeneMate, Lodi, CA; C-3310-1 UltraFlux Dome Cap PCR Tubes) with dome caps. One μL of template DNA was added to each tube and spun briefly in a microfuge to bring down all liquid to the bottom of the tube. All samples were kept on ice until ready for the thermal cycler. Negative controls (NTC, no template controls) were performed with each run to check for contamination and consisted of 1 μL of sterile water added in place of the 1 μL of template DNA to the 19 μL of master mix. Thermal cyclers (Veriti Thermal Cycler or ProFlex Thermal Cycler, Applied Biosystems Inc.) were programmed with the following amplification parameters: 95C for 1 min (1X); 95C for 20 s, 55C for 20 s, and 72C for 90 s (30X); 72C for 7 min (1X); 4C hold (1X). The extension time listed here (90 s @72C) was for amplification of the full-length 16S gene (primed with the 27F and 1492R primers; see Table 6). See Appendix A for a sample Master Mix preparation and a calculation of final concentrations of all PCR components.

F. Agarose Gel Electrophoresis.

Agarose gel electrophoresis was routinely employed to assess both purity and size of amplicons generated by PCR. One percent (w/v) agarose gels were made in 1X TAE (40 mM Tris - Acetate, 1 mM EDTA) buffer according to standard protocols (Maniatis reference). SYBR Safe (10,000X concetrate in DMSO [Invitrogen]) was incorporated at a final concentration of 1X in 10-cm gels according to manufacturer's recommendation (https://www.thermofisher.com/order/catalog/product/S33102). Typically 10 µL of amplicon was mixed with 2 µL of 6X Loading Dye (0.25% (w/v) Bromophenol Blue, 0.25% (w/v) Xylene Cyanol, 30% (v/v) Glycerol) and all 12 µL loaded into a single lane. DNA size markers were always run adjacent to experimental samples (HiLo DNA Ladder, Minnesota Molecular; http://www.mnmolecular.com) to permit estimation of amplicon length. Gels were run for 45-60 minutes at 100 V and imaged immediately with UV light using a Kodak Imager System (GL100).

G. Automated Sanger Dideoxy Sequencing.

Only amplicons that were judged by agarose gel electrophoresis to be both clean (*i.e.*, a single band) and of sufficient quantity (based on intensity of the band) were processed for DNA sequence analysis. If there was more than 1 µg of an amplicon in a lane then that sample was diluted accordingly for DNA sequence analysis (typically between 10- and 100-fold depending on intensity of the band).

All dilutions were done with sterile deionized water. Samples submitted for sequencing contained 1 μL of amplicon (or diluted amplicon), 1 μL of forward or reverse primer (10 μM stock), and 8 μL of sterile deionized water. Samples were always subjected to sequencing in both the forward and reverse directions. Sequencing was performed using the BigDye Terminator Cycle Sequencing Kit Version 3.1 (Applied Biosystems Inc., Foster City, CA 94404; https://tools.thermofisher.com/content/sfs/manuals/cms_081527.pdf) following the manufacturer's instructions with the exception that we routinely ran 1/16 reactions. Cleanup was performed using an EdgeBio Performa DTR Gel Filtration Cartridges (Gaithersburg, MD; https://www.edgebio.com). The samples were analyzed using an ABI3130 Genetic Analyzer from Applied Biosystems (Foster City, CA) using a 36-cm column array and NANOPOP^{TM}7 polymer (MCLAB, South San Francisco, CA 94080, NP7-100; http://www.mclab.com). Sequence calls were made using the KB basecaller.

H. Metagenomic DNA Extractions

Individual hot dogs were washed with 25 mL of sterile deionized water and filtered through a 0.45 μM Nalgene filter units (115 mL, cellulose nitrate, Part No. 121-0045, Thermo Scientific) using vacuum. Any bacteria in these washings should have been trapped on the membrane. Membranes were cut up into small pieces (< 0.5 cm) under sterile conditions and placed into a sterile 50-mL conical plastic tube. Total DNA was extracted from these membranes using

the EpiCentre Metagenomic DNA Isolation Kit for Water (EpiCentre Technologies, Madison, WI; Part No. MGD08420) according to the manufacturer's instructions with the exception that precipitated DNA was dried for 5 minutes in a SpeedVac (Savant Instruments, Saroor Nagar, Hyderabad, India; SC110 http://www.savantindia.in/index.php) instead of air drying. Individual pellets were resuspended in 50 µL of TE (10 mM Tris, 1 mM EDTA, pH 8.0) and 2 µL used to assess DNA concentration and purity on a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific). All metagenomic DNA samples were stored at -20C.

I. Subcloning of 16S rDNA of Metagenomic Samples

Metagenomic DNA samples isolated from hot dogs purchased from the same site were pooled (2 µL of each) and an aliquot (1 µL of 6 µL total) of this pooled sample was amplified for full-length 16S rDNA as described in Section E (above) using the 27F and 1492R primers (Table 6). Correct amplicons (*ca.* 1500 bp) were verified by agarose gel electrophoresis and then ligated with a linearized pMiniT vector (see Appendix B) using the NEB PCR Subcloning Kit (NEB #E1203S; New England Biolabs, Ipswich, MA). Recombinant plasmids were transformed into chemically-competent *E. coli* cells (NEB 10-beta) as described by the manufacturer. Aliquots (50 µL) of each reaction were spread onto 10 LB-AMP (100 µg/mL) plates and incubated overnight at 37C. Colonies were picked randomly from each plate, regrown on LB-AMP plates, and generated as amplicons by Colony PCR as described in Section J.

J. Colony PCR

E. coli colonies were picked from a LB-AMP plates using a sterile inoculation loop and subcultured into new gridded LB/AMP plates. The overnight subculture was then introduced into a PCR tube containing 100 µL of 5% w/v Chelex prepared in 100mM of Tris Buffer at pH 11. The tubes were vortexed and boiled in a heat block for 10 mins. Then they were spun at 20,000 rpm for 2 minutes. 20µl of the supernatant was extracted and 1µl was used for colony PCR. 1µl of pMiniT F and pMiniT R (Appendix B) primers were used. The PCR tubes with the bacterial colonies were placed into the thermocycler and ran under the colony PCR settings. The PCR amplicons were then electrophoresed on an Agarose gel (see Materials and Methods Section F). Bands visible on the gel were screened and prepared for DNA sequencing analysis as explained in section G of Materials and Methods.

K. Next Generation DNA Sequencing of Metagenomic Samples

For the Next Generation Sequencing section, experimental and control groups were formed using 2 µL of the filter extractions and pooled into three different Eppendorf tubes. The experimental groups were labeled E_1, E_2 and E_3 while the controls were labeled C_1, C_2 and C_3. The experimental contained all three hot dogs from twenty-six neighborhoods and the controls included three hot dogs from five different packaged brands. NanoDrop readings were done to the six NGS samples and the data was created. The six samples were packaged, with

their caps wrapped in Parafilm, and sealed in a ZipLock bag. Samples were packed on dry ice and shipped to GENEWIZ, Inc (South Plainfield, NJ).

MetaVx™ 16S rDNA next generation sequencing library preparations and Illumina MiSeq sequencing was conducted at GENEWIZ, Inc. (South Plainfield, NJ, USA). DNA samples were quantified using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) and DNA quality was checked on a 0.6% (w/v) agarose gel. Sequencing libraries were constructed using a MetaVx™ 16S rDNA Library Preparation kit (GENEWIZ, Inc., South Plainfield, NJ, USA). Briefly, 50 ng DNA was used to generate amplicons that cover the V3, V4 and V5 hypervariable regions of bacteria and Archaea16S rDNA. Indexed adapters were added to the ends of the 16S rDNA amplicons by limited cycle PCR. Sequencing libraries were validated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and quantified by Qubit and real time PCR (Applied Biosystems, Carlsbad, CA, USA). DNA libraries were multiplexed and loaded onto an Illumina MiSeq instrument according to manufacturer's instructions (Illumina, San Diego, CA, USA). Sequencing was performed using a 2 x 250 paired-end (PE) configuration; image analysis and base calling was conducted by the MiSeq Control Software (MCS) on the MiSeq instrument. Taxonomy information was carried out on Illumina Basespace cloud computing platform (see Appendix C). All NGS data will be deposited and archived in GenBank (www.ncbi.nlm.nih.gov).

L. Bioinformatic Analyses

All sequencing data generated locally was first viewed and edited using 4Peaks software (http://4peaks.en.softonic.com/mac). This software was also used to directly submit sequences for searching against Genbank using the BLASTn algorithm. BLAST2Seq algorithm was also used to produce alignments between overlapping sequences and to help resolve inconsistencies between forward and reverse sequencing reads. BLASTn searches, unless otherwise specified, were done using standard default values and matches with an e (expect) value of $< 10^{-4}$ was considered as a match. In addition, the Next Generation Sequencing data analysis was carried out by Genewiz (South Plainfield, NJ) on the Illumina Basespace cloud computing platform.

Results

Quantitation of Culturable Bacteria from Field and Control Hot Dogs

A. Site Selection and Sampling.

The hot dog carts were randomly selected from either Manhattan or the Bronx and location was indicated as listed in Table 3 and Figure 1 below. Since we did not want to identify individual carts, and wanted the sampling to be anonymous, we did not report exact locations but limited the location identification to specific neighborhoods. Hot dog sampling occurred between the months of March and August in 2015, and a total of 26 neighborhoods were sampled.

As detailed in Materials & Methods, three hotdogs were purchased from each vendor and we requested no rolls and no condiments. Typically, collections were done with three individuals in the field: one driver and two to purchase and process samples at each site. Hot dogs were typically received on a paper plate (napkins) or in aluminum

foil. Temperatures were recorded as soon as possible after purchase (usually <1 minute) and recorded. Samples were then transferred to labelled, sterile plastic bags, sealed, and stored in a cooler for transport back to the laboratory. The first collection through Manhattan covered 7 neighborhoods and 1 in the Bronx (Morris Park). The following two collections added 18 additional neighborhoods in Manhattan, from the southern most tip to the upper west side (Table 3 and Figure 1).

List of Manhattan Neighborhoods Sampled		
Site Number	**Manhattan Neighborhood**	**Collection Date**
1	Chelsea	3/27/15
2	Flatiron District	3/27/15
3	Murray Hill	3/27/15
4	Midtown (Penn Station)	3/27/15
5	Central Park	3/27/15
6	Morris Park (Bronx)	3/27/15
7	Lower East Side	3/27/15
8	East Village	3/27/15
9	Financial District	4/11/15
10	Two Bridges	4/11/15
11	Chinatown	4/11/15
12	Soho	4/11/15
13	Tribeca	4/11/15
14	West Village	4/11/15
15	Flatiron District	4/11/15
16	Civic Center	4/11/15
17	Hell's Kitchen	8/29/15
18	Upper West Side	8/29/15
19	Lincoln Square	8/29/15
20	Clinton	8/29/15
21	Turtle Bay	8/29/15
22	Lenox Hill	8/29/15
23	Upper East Side	8/29/15
24	Yorkville	8/29/15
25	Kips Bay	8/29/15
26	Alphabet City	8/29/15

Table 3: Random sampling of hot dogs were carried out in 26 separate neighborhoods from two New York boroughs. The site number indicates the order in which the collection was carried out. The Flatiron District was sampled twice, in two separate locations (Sites 2 and 15). All sites were in Manhattan except for Site #6.

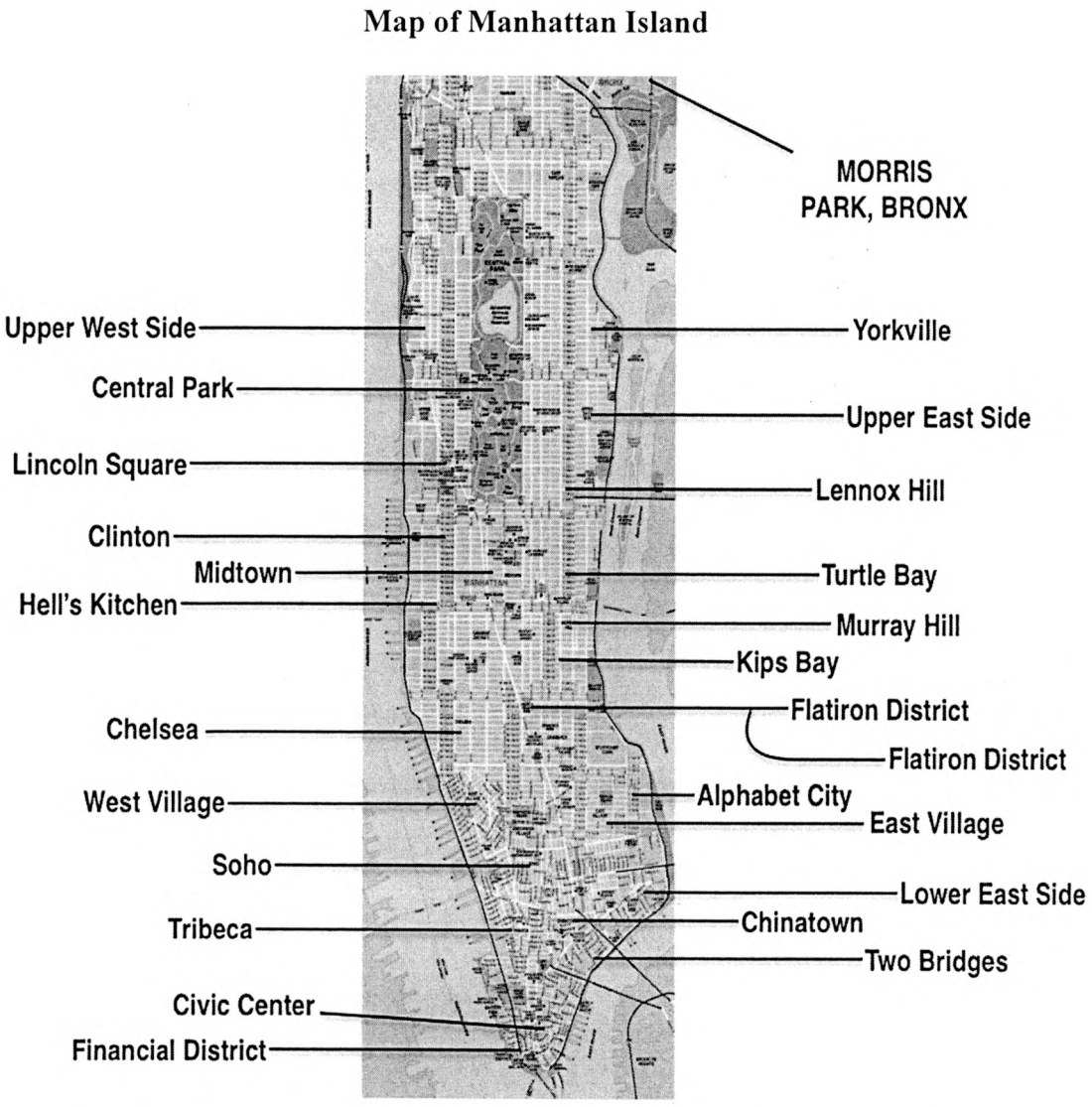


Figure 1: A map depicting the locations of hot dog sampling throughout two New York boroughs.

B. Determination of Culturable Bacteria on Hot Dogs.

Once in the lab the hot dogs were each rinsed with 25 mL of sterile deionized water in a sterile, sealed sample bag (Figure 2). In the laboratory, all procedures were performed under a laminar flow hood unless otherwise indicated.

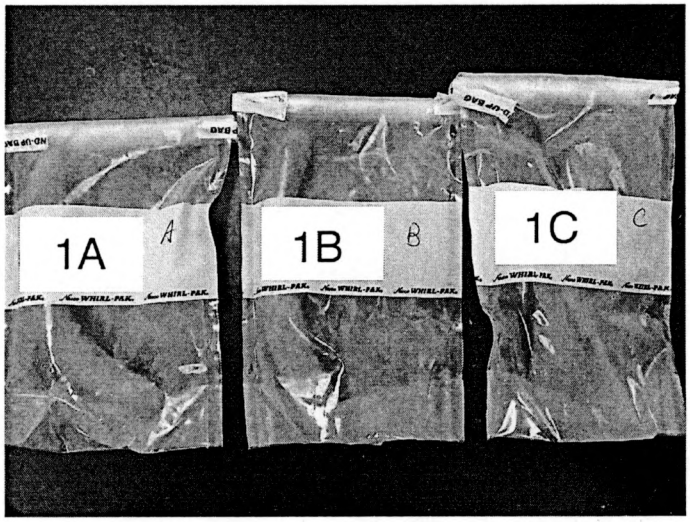


Figure 2: Sample of individual hot dogs in sterile bags prepared for rinsing. Number indicates the site number; letters represent samples from site. Each bag was opened under sterile conditions, 25 mL of sterile DI water was added, then bag was sealed and shaken for 30 seconds. The sealed bag was then re-opened and the 25 mL of washings recovered with a sterile 25 mL pipette.

From the 25 mL of washings recovered from each hot dog, a total of 1.5 mL was set aside for standard microbiological testing. From this, 100 µL was spread in triplicate on LB, LB-AMP, LB-CHL, LB-KAN, and LB-TET plates, producing a total of 15 plates from each sampled site. All platings were done under a sterile hood, and samples were allowed to dry for 30 minutes, with lid on and plate in upright position, before transfer to a 37C incubator. Plates were checked after 24 hours, and colony numbers scored and

plates photographed after 48 hours of growth. A sample plate can be seen in Figure 3. Results from the analysis of all 26 sites is tabulated in Table 4.

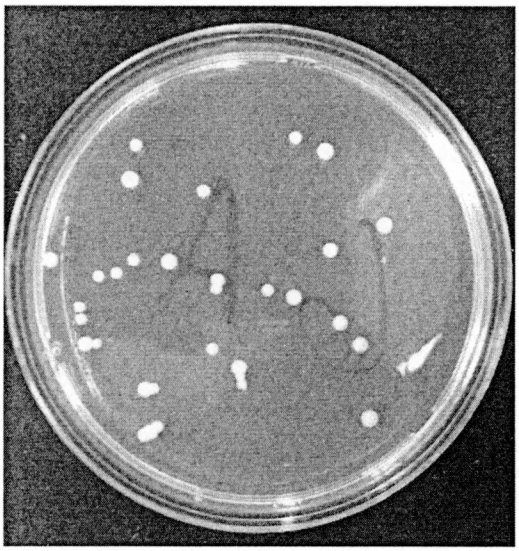


Figure 3: Sample Petri dish (LB) plated with 100 μL of washings from Site 6 (Morris Park in the Bronx, NY).

Table 4 demonstrates that a majority of sites sampled were positive for culturable bacteria (18/26 or 69.2%). Conversely, 30.7% of our sites sampled had no culturable bacteria under our assay conditions. This does not mean that they were entirely free of microbes; just that under our particular culture conditions (LB medium, 37C, aerobic environment, *etc.*) we did not see any growth. Also of note is the presence of antibiotic resistant bacteria detected at nearly 20% of sites sampled (5/26). In fact, over 7% of all sites were positive for bacteria that were resistant to multiple antibiotics (2/26). The colony numbers listed in Table 4 are only for colonies appearing on LB plates without antibiotics.

Neighborhood	Site #	Colony Count on LB Plates	Mean Temp Fahrenheit	CFU's per hot dog	Antibiotic Resistance
Chelsea	1	1	121.7 F	83	-
Flatiron District	2	0	138.7 F	0	-
Murray Hill	3	0	149 F	0	-
Midtown	4	0	128.7 F	0	-
Central Park	5	8	118.7 F	667	-
Morris Park (Bronx)	6	29	133.3 F	2417	-
Lower East Side	7	0	166 F	0	-
East Village	8	6	113 F	500	-
Financial District	9	1	143.7 F	83	-
Two Bridges	10	2	154.7 F	167	-
Chinatown	11	3	125 F	250	•[1]
Soho	12	1	129.8 F	83	-
Tribeca	13	4	130.7 F	333	-
West Village	14	4	136.3 F	333	-
Flatiron District	15	3	130.7 F	250	-
Civic Center	16	2	125.3 F	167	-
Hell's Kitchen	17	0	154.3 F	0	-
Upper West Side	18	1	134.3 F	83	-
Lincoln Square	19	0	145 F	0	-
Clinton	20	2	143.3 F	167	-
Turtle Bay	21	248	128.7 F	20667	•[1]
Lenox Hill	22	46	125 F	3833	•[1]
Upper East Side	23	0	142.3 F	0	-
Yorkville	24	1504	115.3 F	125333	•[10]•[2]•[409]•[1]
Kips Bay	25	0	110 F	0	-
Alphabet City	26	631	126.6 F	52583	•[9]•[28]

Table 4: CFU (colony forming units), serving temperatures, and antibiotic resistance in culturable bacteria found at all sampling sites. CFU's are calculated to be the mean number of bacteria isolated per hot dog, averaged for 3 hot dogs from each site. Temperature (^{o}F) is the mean of three separate readings from hot dogs purchased from each site. CFU's were calculated by colony growth on LB plates cultured at 37C for 48 hours. Black dot (•) indicates Ampicillin resistance, green dot (•) indicates Chloramphenicol resistance, blue dot (•) indicates Kanamycin resistance, and red dot (•) indicates Tetracycline resistance. A dash (-) indicates that no antibiotic resistant colonies

were detected in that triplicate sample. Superscript indicates the exact number of colonies growing on the individual antibiotic plates.

By using the total colony count on LB plates for the washings from each hotdog, and calculating the dilution factor when plating only 100 μL of the 25,000 μL in each washing (1/250), we can calculate CFU's for each sampled hot dog. This is an important value since it allows us to compare total numbers of (culturable) microorganisms associated with each hot dog between sites. And since hot dogs were kept intact, and not punctured or cut to expose internal contents, then this count represents only those culturable bacteria associated with the surface, or biofilm, of each sample. As can be seen in Table 4, these numbers varied widely, from 0 to more than 1.25×10^5 CFU's per hot dog. (Mean = 8,000; S.D. = 26,274; N = 26).

Quantitation of culturable bacteria associated with store-bought controls was also analyzed. Controls were performed as described in Materials & Methods. Briefly, 5 different commercial brands of all beef hot dogs were purchased locally. All were well within their expiration dates. All samples were processed in the laboratory identically to field-collected hot dogs with the obvious exception that these samples were not heated in the field by mobile vendors. The data from this analysis is presented in Table 5 below.

Of the five brands used for the store-bought controls, only Hebrew National showed absolutely no culturable bacteria in our assay. Of the remaining four brands, they ranged from 4.2×10^2 to 2.5×10^3 CFU's per hot dog.

Brands	ID #	Colony Count	CFU's per Hot Dog	Antibiotic Resistance
Oscar Mayer	1	5	417	-
Ball Park	2	30	2500	-
Nathan's	3	6	500	-
Hebrew National	4	0	0	-
Sabrett	5	25	2083	-
Water Control Sites 1-8	6	0	0	-
Water Control Sites 9-16	7	0	0	-
Water Control Sites 17-26	8	0	0	-
Water Control Brands 1-4	9	0	0	-
Water Control Brand 5	10	2	167	-

Table 5: Store-bought commercial controls. Samples were purchased locally and were all well within expiration dates. Colony counts, CFU calculations and antibiotic resistance testing were identical to testing of field-collected hot dogs (Table 4). All water controls were conducted as described in Materials & Methods. All water controls were negative except for the water control for the last brand sampled (Sabrett's).

In contrast to the field-collected samples, none of the controls had any bacteria that were resistant to the antibiotics tested in this study. And both the mean and maximum of CFU's per hot dog in the control (X = 1,100; max. = 2,500) was significantly below both the mean and maximum values seen in the field-collected samples (X= 8,000; max. = 125,333). Water controls for all analyses were conducted as described in Materials & Methods. All water controls were negative except for the water control for the last brand sampled (Sabrett's). Five empty plates (LB, LB-AMP, LB-CHL, LB-KAN, and LB-TET) were placed in a 37C incubator during every sampling test to determine the sterility of the LB-agar. All of these controls always resulted in zero growth; thus, no tables or figures were created.

C. Correlation of Temperature and CFU's from Field-Collected Hot Dogs

In an effort to determine if the serving temperature of on-street hot dogs influenced the presence of culturable microorganisms we compared experimentally determined temperatures (taken without touching samples by using an infrared thermometer shortly after triplicate samples were purchased) with the resulting CFU's measured for washings. As shown in Table 4, we see that samples that had the largest CFU's were often inversely correlated with temperatures. For example, the highest measured CFU was Site 24 (Yorkville) with 1.25×10^5 CFU's per hot dog. The temperature of this sample was 115.3 F, which is far below the NYC Health Code recommendation of 145 F (Burt, 2003). In fact, if we use this 145 F standard as a cutoff point, we see that only 5 out of 26 sites (19.2%) had a serving temperature of 145 F or higher (Table 4). Likewise, 21 of the 26 sites (80.7%) had a temperature lower than 145 F. For the group above 145 F, four values were 0 and only one had measurable CFU's (167 for Site 10, Two Bridges). **That was equivalent to a mean CFU of 33.9 per hot dog for the above 145 F group**. For the group below 145 F, 17 out of 21 sites had positive CFU's (81%) and 4 sites had no measurable CFU's (19%). **That was equivalent to a mean CFU of 9,897 per hot dog for the below 145 F group.** The above 145 F group had a mean temperature of 153.8 F with a S.D. of 7.91; the below 145 F group had a mean temperature of 128.6 F with a S.D. of 9.51. Another way to contrast this dichotomy in these two populations is to look at total CFU's generated in this study. If we sum all the CFU's generated (for all 26 sites) we see that only 0.08% are associated

with hot dogs served at 145 F or above, while a staggering 99.92% are associated with hot dogs served at temperatures below 145 F.

A scatter plot (Figure 4) graphically illustrates this inverse relationship. Above 145 F there are very few CFU's per hot dog. However, below 145 F the CFU's increase dramatically as the temperature lowers showing a clear trend to more bacteria in hot dogs served at these substandard temperatures. It is important to note that the Y-axis scale is logarithmic, which both expands the lower values and compresses the larger data in this graph.

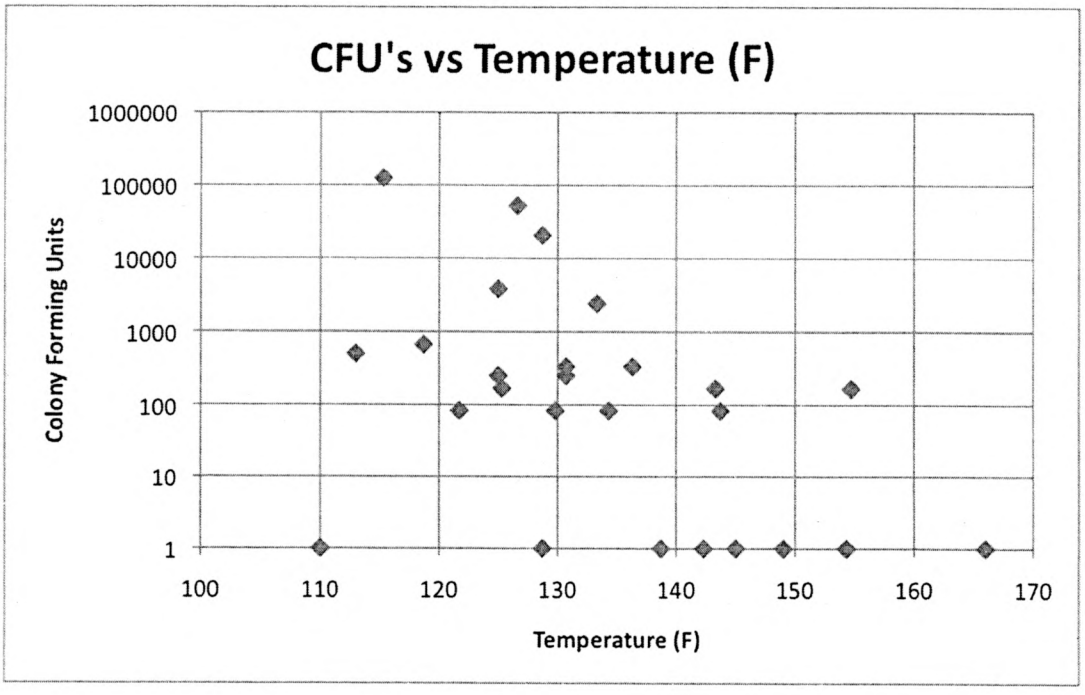


Figure 4. Scatter plot of data from all 26 sites correlating serving temperature of hot dogs (mean of 3 readings in degrees F) with the Colony Forming Units (CFU's) per hot dog.

Molecular Identification of Bacterial Colonies Using 16S DNA Barcoding

A. Colony PCR.

In an attempt to qualitatively identify bacterial colonies isolated by washing of both field-collected and control hot dogs, we isolated individual colonies from each plate that produced CFU's in this experiment. On plates where large number of colonies were generated, an attempt was made to sample representative colonies based on morphology, color and other phenotypic characteristics. Individual picked colonies were re-streaked onto fresh LB plates and incubated overnight at 37 C to ensure a pure, single colony was isolated. For antibiotic-resistant colonies, picked colonies were streaked onto fresh LB plates containing the appropriate antibiotic. Once colonies were re-grown, a single isolated colony was picked and total genomic DNA (gDNA) was isolated using the InstaGene Matrix protocol (Bio-Rad, Hercules, CA). Isolated gDNA was quantified and purity was estimated using UV spectroscopy on a NanoDrop ND-1000. Typically, 1 μL of gDNA was used in a PCR reaction to amplify the prokaryotic 16S locus using the 27F and 1492R universal primer set (Chen, 2015; see Table 6). A typical PCR reaction and mastermix calculation can be found in Appendix A. After amplification, amplicons were evaluated for size, purity, and amount by visualizing 10 μL of the 20 μL PCR reaction on a 1% (w/v) agarose gel incorporating SYBR Safe for imaging DNA. Figure 5 illustrates bacterial gDNA precipitated during this protocol.

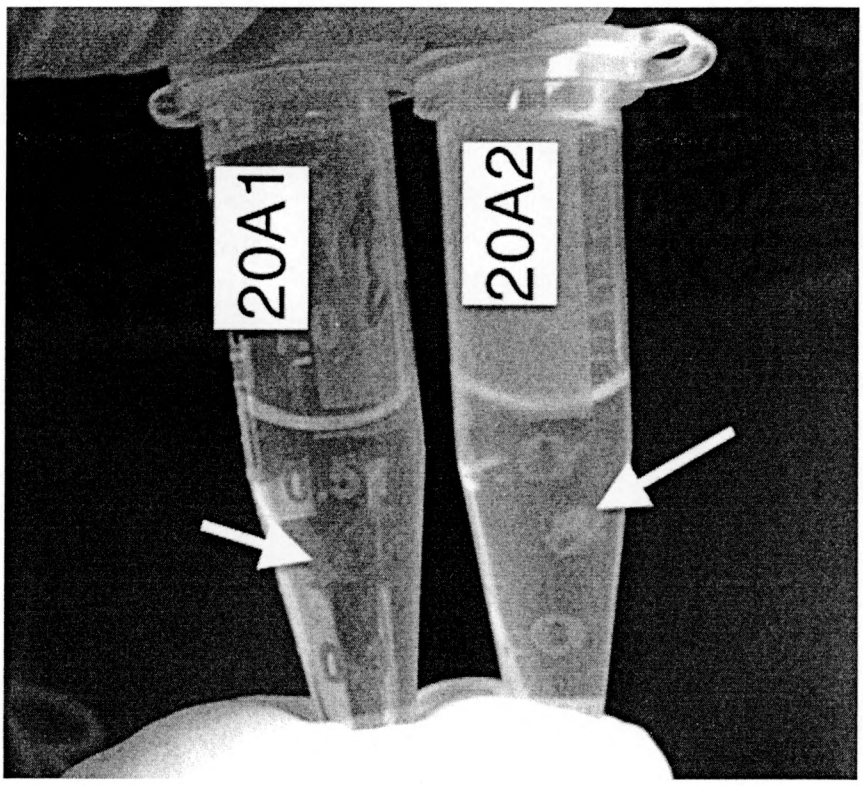


Figure 5: Sample image shows the precipitation of gDNA (see arrows) during the of Colony DNA Isolation protocol.

Name of Sequences	Sequences 5' to 3'
27F	AGAGTTTGATCMTGGCTCAG
338R	GCTGCCTCCCGTAGGAGT
$349F_1$	GYGCASCAGKCGMGAAA
$349F_2$	GYGCASCAGKCGMGAAT
534R	ATTACCGCGGCTGCTGG
515F	GTGCCAGCMGCCGCGGTAA
806R	GGACTACVSGGGTATCTAAT
967F	CAACGCGAAGAACCTTACC
1048R	CGRCRGCCATGYACCWC
1391F	TGYACACACCGCCCGTC
1492R	GGCTACCTTGTTACGACTT

Table 6: List of the 11 primers used in this project. Primers typically target the conserved regions of the 16S locus, with F and R referring to forward and reverse, respectively. For amplification of the nearly full-length 16S locus we used 27F and 1492R primer set. These primers were also utilized for sequencing of amplicons generated. Internal primers were sometimes utilized for sequencing through difficult templates. Some primers contain degeneracies based on IUPAC code (R = A or G; Y = C or T; S = G or C; W = A or T; K = G or T; M = A or C; and V = A, C or G (not T)).

Figure 6 graphically illustrates the positions of these primers relative to the prokaryotic 16S locus. The gene is divided into both conserved and variable regions. PCR and sequencing primers are typically anchored in conserved regions. And amplicons copy through variable regions which allow one to use this locus for barcoding since these variable regions are often unique to particular species.

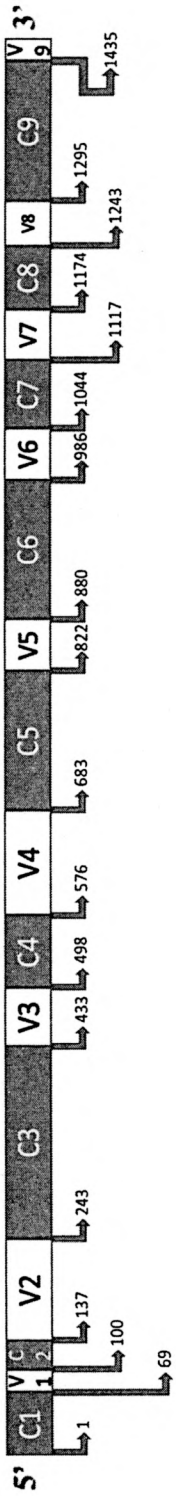


Figure 6: Variable (V) and conserved (C) regions of the prokaryotic 16S rDNA gene. Gene is 5' to 3', left to right. Numbers indicate nucleotide positions where either variable or conserved regions begin and end.

Amplification of the prokaryotic 16S locus using our typical 27F and 1492R primers set would be expected to generate an amplicon that was 1,465 bp (base pairs) long. The 27F primer would bind in the C1 region and the 1492R primer would bind just to the right of the V9 region, in a conserved region near the 3' end of this locus. Thus, this 1465 bp amplicon would contain all 9 variable regions of this gene. Sequencing through the entire amplicon will provide multiple opportunities to uniquely identify a microorganism based on this DNA barcode.

Figure 7 illustrates a typical agarose gel generated from the PCR amplification of the 16S locus from the colony PCR protocol. Each lane represents a different colony isolated from experimental washings. The amplicon size is typically found between the 1550 bp and 1400 bp bands of our standard ladder (Hi-Lo ladder, Minnesota Molecular) which presents as a closely spaced doublet band in the middle of the HiLo DNA ladder. Some amplifications were so robust that we had to dilute the PCR product 10-fold or 100-fold prior to DNA sequence analysis. Not shown on this gel are positive and negative controls. Typically both were done with every batch of colony PCR reactions. Samples that amplified poorly, or not at all, we subjected to a second round of PCR amplification.

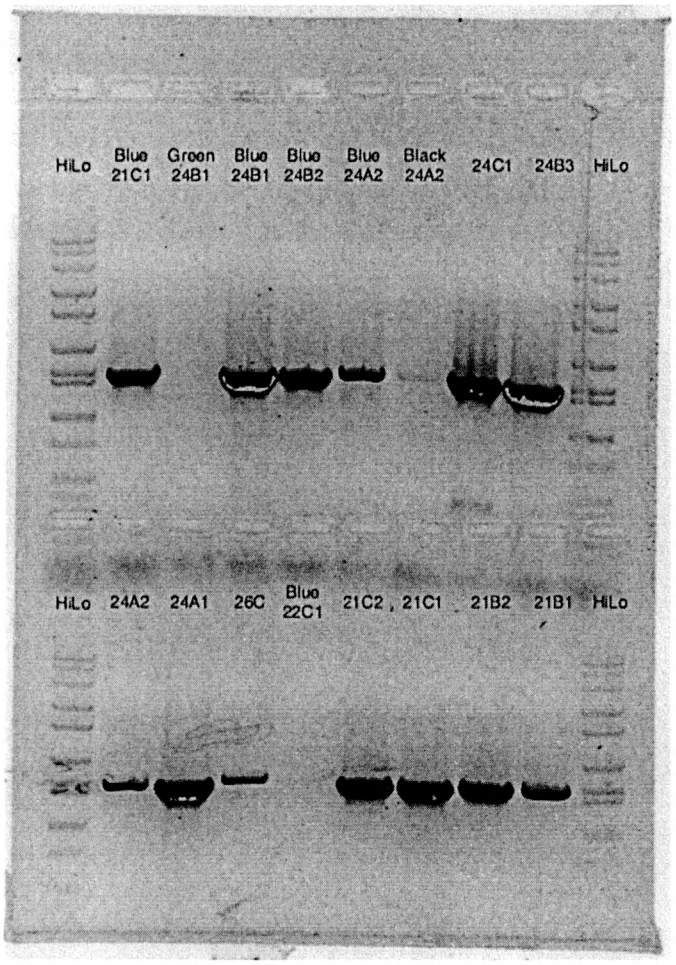


Figure 7: Inverse image is of a 1% (w/v) agarose gel, stained with SYBR Safe. DNA size markers (HiLo) are present in all 4 end lanes. Internal 16 lanes contains 10 μL of a PCR reaction amplifying the 16S prokaryotic locus. Numbers above lanes indicate site number, letters designate which triplicate hot dog (A, B, or C) from each site, and the last number indicates which clone isolate this was from LB plates. Black, blue, green, and red refers to colonies that were either isolated by growth on ampicillin, kanamycin, chloramphenicol, or tetracycline plates, respectively. Amounts of product varied, but typically a very strong band of *ca.* 1465 bp was generated. Samples that were amplified cleanly (i.e., just a single band of the correct size) were directly sequenced using Automated Sanger Dideoxy protocol as described in Materials & Methods.

Those amplicons that were judged clean (single, strong band of the correct size) was subjected to automated Sanger Dideoxy sequence analysis as described in Materials & Methods. Both forward and reverse sequencing reactions were performed on all samples, using the same PCR primers that generated the original amplicon. A typical

electropherogram generated by this process is shown in Figure 8. This is a small representative portion of one of the two reactions for each sample sequenced. Although the automated base calling done by the ABI 3130 software was generally reliable, every sequence was reviewed for anomalies and sequences edited manually in those cases.

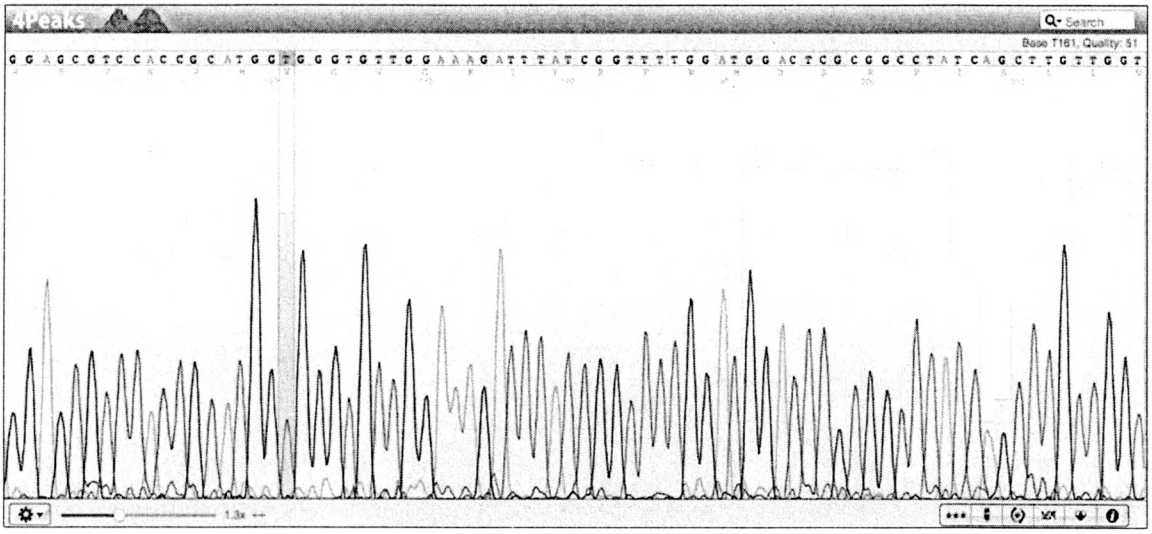
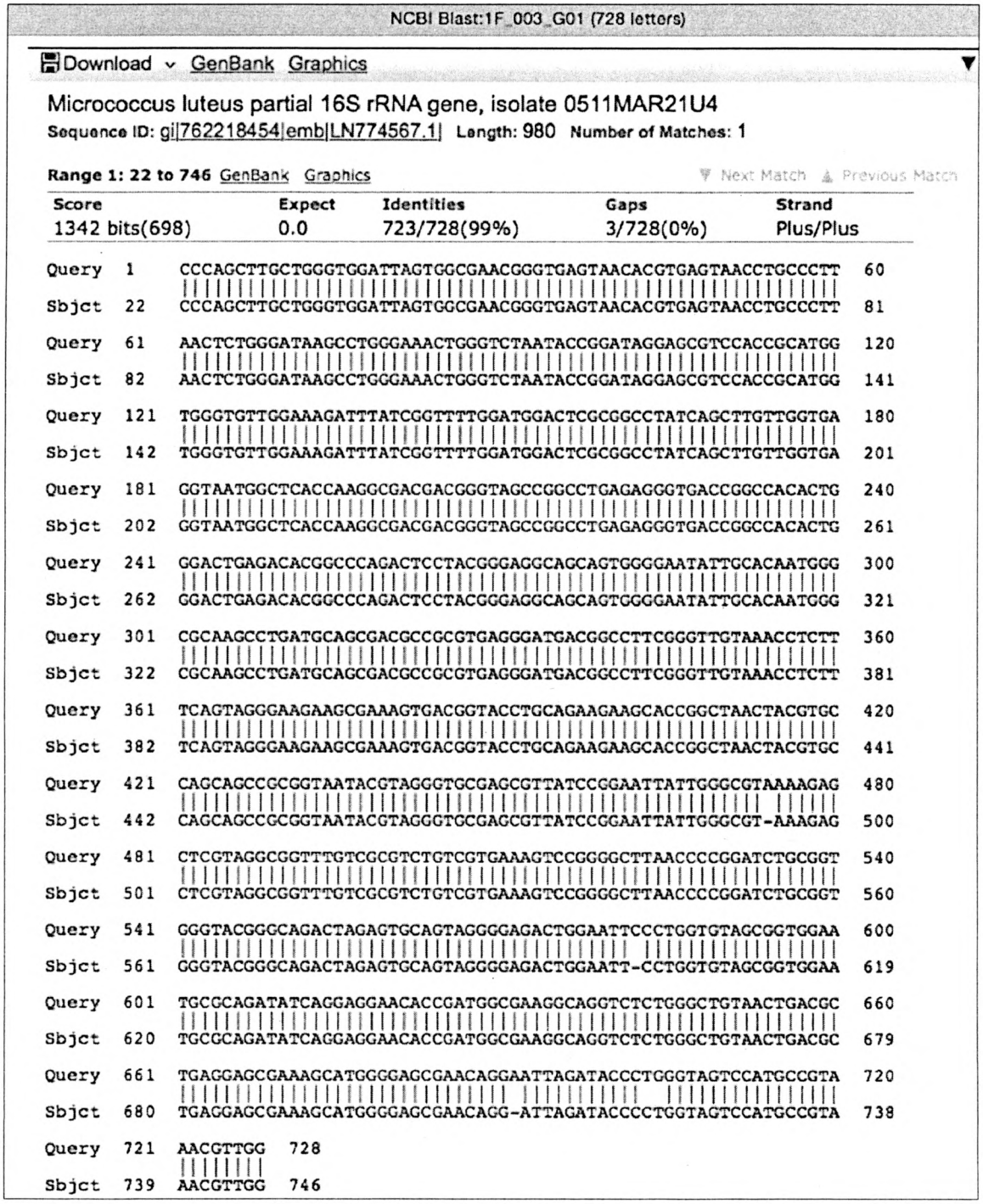


Figure 8: A portion of an electropherogram generated by direct DNA sequencing our our Colony PCR amplicons in this study. Electropherograms were viewed and edited using 4 Peaks software (insert URL here). The gray histogram in the background visually shows the quality read score (ratio of fluorescence signal strength to the background fluorescence; the higher the bar, the better the quality of the read). All sequences were run on an Applied Biosystems 3130 Genetic Analyzer using a 36-cm column array and NANOPOP-7^{TM} polymer.

All sequence data was subsequently searched using the BLASTn algorithm of GenBank (http:\\www.ncbi.nlm.gov). Unless other specified, standard defaults were used and both the forward and reverse sequences of each amplicon were searched separately. The top 5 hits, and their alignments, were examined and the likely identity of our unknown was confirmed. If our e value was 10^{-4} or less, then this was scored as a match at the species level. Generally, we used both forward and reverse sequences to confirm

identity. Since each Sanger dideoxy run can only reasonably sequence *ca.* 700 bp into any given amplicon, it is possible to get different identities by searching forward and reverse sequences. In this case, it may be possible to join the forward with the reverse complement of the reverse sequence, if there is sufficient overlap at the ends, to make one long, assembled contig. Then search this against the Genbank database to find the best match. In some cases where it was not possible to join forward and reverse sequences due to lack of overlap at 3' ends, it was possible to utilize other internal 16S primers to generate good data internally that will overlap and permit full assembly of the contig. Figure 9 shows a BLASTn search of an unknown sequence against Genbank and a subsequent alignment to the best match. In this case the e value was 0.0, the homology was 723/728 (99%), with 3 gaps. The quality of the match in this case was very good and this was only done with the forward primer (27F) on the amplicon. A separate analysis of the reverse data also confirmed the identity as *Micrococcus luteus* (not shown). Not all molecular identifications using the 16S locus are this clear cut, but typically we have been able to make a call for most of the 16S sequences generated in this project.

Table 7 complies all identified bacteria from our experiment using the DNA barcoding of the 16S rDNA locus. In cases where there are multiple species listed, these were present separately in the sample and do not imply any ambiguity. In a few cases we have listed identity as "uncultured bacterium" or "unidentifiable." In these cases we did obtain a good match to a record in Genbank but the organism is simply unknown or unidentified at the genus or species level.



NCBI Blast:1F_003_G01 (728 letters)

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Micrococcus luteus partial 16S rRNA gene, isolate 0511MAR21U4

Sequence ID: gi|762218454|emb|LN774567.1| Length: 980 Number of Matches: 1

Range 1: 22 to 746 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1342 bits(698)	0.0	723/728(99%)	3/728(0%)	Plus/Plus

```
Query  1    CCCAGCTTGCTGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGAGTAACCTGCCCTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22   CCCAGCTTGCTGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGAGTAACCTGCCCTT  81

Query  61   AACTCTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATAGGAGCGTCCACCGCATGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  82   AACTCTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATAGGAGCGTCCACCGCATGG  141

Query  121  TGGGTGTTGGAAAGATTTATCGGTTTTGGATGGACTCGCGGCCTATCAGCTTGTTGGTGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  142  TGGGTGTTGGAAAGATTTATCGGTTTTGGATGGACTCGCGGCCTATCAGCTTGTTGGTGA  201

Query  181  GGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  202  GGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTG  261

Query  241  GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  262  GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGG  321

Query  301  CGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  322  CGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTT  381

Query  361  TCAGTAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  382  TCAGTAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGC  441

Query  421  CAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTATCCGGAATTATTGGGCGTAAAAGAG  480
            |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  442  CAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTATCCGGAATTATTGGGCGT-AAAGAG  500

Query  481  CTCGTAGGCGGTTTGTCGCGTCTGTCGTGAAAGTCCGGGGCTTAACCCCGGATCTGCGGT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  501  CTCGTAGGCGGTTTGTCGCGTCTGTCGTGAAAGTCCGGGGCTTAACCCCGGATCTGCGGT  560

Query  541  GGGTACGGGCAGACTAGAGTGCAGTAGGGGAGACTGGAATTCCCTGGTGTAGCGGTGGAA  600
            |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  561  GGGTACGGGCAGACTAGAGTGCAGTAGGGGAGACTGGAATT-CCTGGTGTAGCGGTGGAA  619

Query  601  TGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCTGTAACTGACGC  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  620  TGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCTGTAACTGACGC  679

Query  661  TGAGGAGCGAAAGCATGGGGAGCGAACAGGAATTAGATACCCTGGGTAGTCCATGCCGTA  720
            |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  680  TGAGGAGCGAAAGCATGGGGAGCGAACAGG-ATTAGATACCCCTGGTAGTCCATGCCGTA  738

Query  721  AACGTTGG  728
            ||||||||
Sbjct  739  AACGTTGG  746
```

Figure 9: BLASTn search and alignment of unknown 16S amplicon (forward direction) using the NCBI (National Center for Biotechnology Information) database. Unknown is identified as *Micrococcus luteus*.

It should be noted that the list of bacterial species in Table 5 (33 different species) does not represent every bacterial colony found growing on LB plates, but does include all colonies found growing on antibiotic containing plates. For example, site 26 (Alphabet City) contained over 630 bacteria on LB plates but since these colonies all looked phenotypically very similar, only a few representative bacterial colonies were sequenced and identified. But two other plates from this site (kanamycin and tetracycline) that had growth were tested separately. So, two of the three species found from site 26 were obtained from LB plates (*Lactobacillus sakei* and *Pseudomonas psychrophila)*, with the third colony (*Carnobacterium maltaromaticum*) found on both kanamycin and tetracycline plates. In cases where the same identification was made multiple times from one site, it only counted once and was listed only once in Table 7.

Since we specifically were amplifying prokaryotic rDNA it is not surprising that most of the species identified in this list are prokaryotic. The fact that we discovered antibiotic resistant bacteria at five of the twenty-six sites associated with a common food product was somewhat surprising. At the Yorkville site we identified a eukaryotic microorganism, *Candida parapsilosis,* which is a fungus and a concern because it has been identified as a potential human pathogen (Trofa *et al.*, 2008). There had been previous reports of yeast 16S rDNA amplification by these conserved, universal prokaryotic primers (Galkiewicz, 2008).

When comparing the number of colonies between neighborhoods we found that the average LB colony count was ninety-six and the median was two. To identify extreme scores a two-tail t-test was created, and with the criteria set to plus or minus two standard deviations, the only neighborhood that was seen outside of this range was Yorkville.

Yorkville had a z-score of 4.55 which is well above the two standard deviation criteria and thus significant, $p < .05$.

Qualitative Identification of Bacteria Using the 16S rDNA Locus

Neighborhoods	Sites	16S rDNA Identification	Antibiotics Resistance
Financial District	9	*Bacillus megaterium*	-
Two Bridges	10	*Bacillus megaterium*, *Bacillus licheniformis*	-
Chinatown	11	*Bacillus aerophilus*, *Bacillus pumilus*, *Bacillus amyloliquefaciens*	•
Soho	12	*Bacillus amyloliquefaciens*	-
Tribeca	13	*Bacillus licheniformis*, *Bacillus amyloliquefaciens*	-
West Village	14	*Bacillus licheniformis*, *Bacillus amyloliquefaciens*	-
Flatiron District	15	*Bacillus safensis*, *Bacillus licheniformis*, *Bacillus methylotrophicus*	-
Civic Center	16	*Bacillus licheniformis*, *Bacillus subtilis*	-
Upper West Side	18	*Staphylococcus hominis*	-
Clinton	20	Uncultured bacterium	-
Turtle Bay	21	*Kocuria rhizophila*, *Macrococcus caseolyticus*, *Enterococcus faecalis*, *Micrococcus yunnanensis*, *Kocuria rhizophila*	•
Lenox Hill	22	Uncultured bacterium, Unidentifiable, *Kocuria rhizophila*, *Bacillus licheniformis*	•

Yorkville	24	Uncultured bacterium, Unidentifiable, *Kocuria rhizophila,* *Enterococcus faecalis,* *Staphylococcus warneri,* *Moraxella osloensis,* *Arthrobacter sanguinis,* *Bacillus subtilis,* *Bacillus cereus,* *Exiguobacterium marinum,* *Candida parapsilosis*	••••
Alphabet City	26	*Carnobacterium maltaromaticum,* *Pseudomonas psychrophila,* *Lactobacillus sakei*	••

Table 7: A list of bacteria that were identified using Colony PCR and DNA Barcoding at the 16S rDNA Locus.

Qualitative Identification of Bacteria on Control Plates Using the 16S rDNA Locus

Brand	ID #	16S rDNA	Antibiotics
Oscar Mayer	1	*Bacillus firmus,* *Bacillus megaterium*	-
Ball Park	2	Uncultured bacterium, *Lysinibacillus xylanilyticus,* *Lactobacillus sakei*	-
Nathan's	3	Uncultured bacterium, *Bacillus flexus*	-
Sabrett's	5	*Bacillus endophyticus,* *Bacillus infantis,* *Microbacterium oxydans,* *Bacillus megaterium*	-
Water Control Brand 5	6	*Bacillus megaterium,* *Bacillus flexus*	-

Table 8: A list of bacteria that were identified on Control Plates using Colony PCR and DNA Barcoding at the 16S rDNA Locus.

For the control hot dogs, only one commercial brand (Hebrew National) showed no CFU's with processed washings. The other four brands had anywhere from from 4.2 x

10^2 to 2.5 x 10^3 CFU's per hot dog. Both the mean and maximum of CFU's per hot dog in the control (X = 1,100; max. = 2,500) was significantly below both the mean and maximum values seen in the field-collected samples (X= 8,000; max. = 125,333). In addition to having significantly lower CFU's per hot dog in the control group, they were also less diverse from a qualitative perspective. This group only had 10 different culturable types of bacteria identified, well below the 33 found in the field collected hot dogs. Also in contrast to the field-collected samples, none of these control samples had any bacteria that were resistant to the antibiotics tested in this study.

As mentioned previously, only one of the water controls showed contamination. This was for one of the control group hot dog samples (Sabrett's). There were two different microorganisms detected in this analysis. The most likely source of this contamination is from our distilled water source used on that day. Since no other contamination had ever been detected in any other assays, we assume this was a one-time, and not a recurrent, problem of contamination.

Due to the massive difference between the sample sizes of the control and experimental groups we decided to compare each individual neighborhood to the control sample. The average LB count of the control sample was 13.2 and the standard deviation was 13.37. Four of the eighteen neighborhoods fell outside the range of two standard deviations, which indicates significance, $p < .05$. The four neighborhoods are Alphabet City, Lexon Hills, Yorkville and Turtle Bay. These four neighborhoods are not contiguous but they are all located on the east side of Manhattan.

Discussion

Anyone who has ever stared into a steaming bin of hot dogs on a mobile cart in Manhattan has probably wondered "what's in there besides hot dogs?" And "why does everyone refer to them as dirty water dogs?" These are questions that have long been asked, but have been woefully short on real, experimental data. So, this research project was inspired to address these burning questions related to dirty water dogs. We planned to answer these questions by examining culturable microbes, and to assess, both qualitatively and quantitatively, the microbial population in on-street hot dog water.

The development of our research strategy required that we access the water that the hot dogs were bathed in. Since assaying and testing this water directly was not possible, we devised a strategy that permitted us to indirectly collect this water. When hot dogs are removed from their bath, they have a small amount of liquid adhering to them. When placed in a bun, most of that liquid is absorbed by the bread. So, we requested our hot dogs with no buns, and no condiments so that the water associated with each hot dog was not absorbed by the bread or contaminated by condiments. Vendors usually delivered three plain hot dogs in aluminum foil or a paper tray, and then we quickly transferred them to sterile plastic bags for transport (on ice) back to the laboratory. Typically we would have < 1 mL of liquid from each hot dog, which was supplemented with 25 mL of sterile water to rinse and quantitatively transfer any microorganisms adhering to the hot dog or found in this liquid.

We were also interested in maintaining absolute anonymity of vendors in this study so they were chosen randomly. To this end we only identified local neighborhoods and never recorded any information that could identify an individual vendor or specific

address. Although sites are identified by neighborhood, we do not ascribe any special significance to this location. Our data simply reports on the culturable microorganisms found associated with that anonymous vendor, at a randomly selected located, on that given day. This study was designed to provide a preliminary examination of potential microbial populations associated with on-street hot dog water. This is clearly a complex problem, and the potential sources of environmental contamination are numerous.

With that in mind, we found that detectable levels of culturable bacteria associated with these washings from experimental hot dogs revealed that a majority of the sites sampled were positive for culturable bacteria (18/26 or 69.2%). The numbers of culturable bacteria varied widely, from 0 to more than 1.25×10^5 CFU's per hot dog for the 26 sites sampled (X = 8,000; S.D. = 26,274; N = 26). Conversely, 30.7% of our sites sampled had no culturable bacteria under our assay conditions. This does not mean that they were entirely free of microbes; just that under our particular culture conditions (LB medium, 37C, aerobic environment, *etc.*) we did not see any growth. These settings were chosen since they are considered standard conditions for the culture of microorganisms. In the analysis of our data it is important to note that microbiologists have been aware of what is generally referred to as the "1% problem." This is the idea that less than 1% of microorganisms present in environmental samples will grow in the laboratory under these standard conditions. In fact, this value, when experimentally determined is often far below 1% (Garland et al., 2001; Stewart, 2012). Thus, when interpreting our numbers of culturable bacteria per sample it is prudent to understand that the actual numbers of total microorganisms is likely to be considerably higher.

The presence of antibiotic resistant bacteria detected at nearly 20% of sites sampled (5/26) is of special concern. The proliferation of antibiotic-resistant plasmids among microbes in natural populations has been a growing concern to the medical community (Ventola, 2015). Our discovery that 20% of all sites sampled were positive for antibiotic resistant bacteria, and that over 7% of all sites were positive for multidrug resistant bacteria, is especially alarming. The Yorkville and Alphabet City sites, which accounted for a total of 2,135 colonies (85% of all colonies from all 26 sites), both contained colonies resistant to multiple antibiotics.

Quantitation of culturable bacteria associated with store-bought controls was considerably lower, with values ranging from 0 to 2.5 x 10^3 CFU's per hot dog (X = 1.1 x 10^3 CFU's per hot dog). In contrast to the field-collected samples, none of the controls had any bacteria that were resistant to the antibiotics tested in this study.

Of our 26 sites we identified twenty-two different bacterial species and one yeast, *Candida parapsilosis*. The combined colony count for all the neighborhoods was of 2496 and of those, only forty-two colonies were genotyped by colony PCR. As explained previously, bacterial colonies from each site were grouped by phenotypic similarities and only representative colonies were sequenced. Of the bacterial species identified by sequencing of the 16S locus of isolated colonies, some of the most common bacteria encountered were *Bacillus licheniformis* and *Kocuria rhizophila* (with an N of 8 and 6, respectively). *Bacillus licheniformis* is a spore forming bacteria, leading it to be resistant to various environmental factors (Sun, 2016). *B. licheniformis* is also a soil dwelling bacteria; however, it has been found in bird feathers (Burtt, 2010). This bacteria is closely related to *Bacillus subtilis* and, similarly, it is believed to cause some illnesses (Burtt,

2010). *Kocuria rhizophila* is a bacterium that was first isolated from the leaves of the Cattail (*Typa angustifolia*); however, it is also commonly found in both soil and water (Takarada, 2008). More importantly, it's also been considered a normal component of human skin flora (Takarada, 2008).

In contrast to the twenty-two bacterial species identified from the water associated with on-street hot dogs, control hot dogs contained only five unique bacteria. Two of these are identical to the two bacteria identified from one of our water controls (Control #5 - Sabrett's). Contamination was seen only in this one instance, which was done separately from the processing of all other control hot dogs. Thus, we believe this represents an isolated instance of contamination present on that one day and for that sample. No other contamination of controls was seen in this set of experiments.

Both the washings from the Sabrett's control hot dog and the water control contained *Bacillus megaterium*. *B. megaterium* is also the most common bacteria found in our control hot dog population with four total colonies isolated and genotyped by colony PCR. *B. megaterium* is known for its extremely large size and it is also a very commonly found bacteria (Eppinger, 2011) in the environment. It is also in common use in our department and is frequently used in our microbiology teaching laboratories. Thus, we cannot rule out the possibility that the presence of *B. megaterium* in our controls (especially in this Sabrett's control) might be due to contamination present in the laboratory.

Although the 16S specific primers used in this study are designed to primarily amplify prokaryotic DNA, we have found that we were also able to amplify eukaryotic DNA (Galkiewicz, 2008). This amplification can have occurred in one of two ways.

Some eukaryotic species can use the 8F, 27F, and 1492R primers to amplify the 18S rDNA gene found in the nucleus of some eukaryotes (Galkiewicz, 2008); or the 27F and 1492R primers can amplify the 16S rDNA gene found in the mitochondria of eukaryotic organisms (Yang, 2014). In this case, based on our DNA sequence analysis, we have identified a mitochondrial 16S gene from *Candida parapsilosis*. *Candida parapsilosis* is a eukaryotic fungus from the Candida family, regularly found in human normal flora but recently it has been associated with human fungal infection (Asbeck, 2009). *C. parapsilosis* was also the second most frequent Candida fungal infection recovered in a Neonatal Intensive Care Unit (Asbeck, 2007).

In the twenty-two different species identified in on-street hot dog water are two common pathogenic bacteria, *Staphylococcus hominis* and *Bacillus subtilis*. (See Appendix E for a list of 200 microorganisms that are potentially pathogenic to humans that was used for comparison). *S. hominis* is commonly found on the surface of the skin in humans, and it is rarely pathogenic. However, this bacterium is very opportunistic and can be pathogenic to humans in a hospital setting, especially to those with weakened immune systems (Jiang, 2012). *B. subtilis* is a soil organism that can form spores. Tam (2006) believes that the *B. subtilis* spores enter the human body via ingestion and can grow rapidly in the gastrointestinal tract. Although *B. subtilis* isn't able to survive the stomach acid, its spores, however, have no issue and once in the intestines the microbe can turn pathogenic (Tam, 2006).

The serving temperature of on-street hot dogs correlated with the presence of culturable microorganisms. As shown in Table 4, samples that had the largest CFU's were often inversely correlated with temperatures. The highest measured CFU in our

study was Site 24 (Yorkville) with 1.25 x 10^5 CFU's per hot dog. The temperature of this sample was 115.3 F, which is far below the NYC Health Code recommendation of 145 F (Burt, 2003). In fact, using this 145 F standard as a cutoff point, we see that only 5 out of 26 sites (19.2%) had a serving temperature of 145 F or higher (Table 4). Likewise, 21 of the 26 sites (80.7%) had a temperature lower than 145 F. For the group above 145 F, four values were 0 and only one had measurable CFU's (167 for Site 10, Two Bridges). **This is equivalent to a mean CFU of 33.9 per hot dog for the above 145 F group**. For the group below 145 F, 17 out of 21 sites had positive CFU's (81%) and 4 sites had no measurable CFU's (19%). **This is equivalent to a mean CFU of 9,897 per hot dog for the below 145 F group.** The above 145 F group had a mean temperature of 153.8 F with a S.D. of 7.91; the below 145 F group had a mean temperature of 128.6 F with a S.D. of 9.51. Our preliminary data suggest that the serving temperature may influence the CFU's per hot dog.

Although we attempted to do everything we could to reduce or eliminate possible sources of bacterial contamination in this project, it is possible that some of field-collected samples might have been subject to external contamination. We live in a microbial world, with some recent estimates, using scaling laws, predicting upwards of one trillion unique species of bacteria on our planet (Locey, 2016). Thus, the likelihood of some bacteria from the environment - outside of the hot dog cart - making it's way into our sample is a possibility. Hot dogs from the vendor were usually delivered on aluminum foil or paper plates, neither of which is a sterile surface. ALthough vendors are required to wear gloves, the gloves themselves are not sterile and only some vendors actually wear gloves (Burt, 2009). Gendron and his colleagues tested the transmission of

bacteria from unused paper towels to hands or surfaces and they found that a large community of culturable bacteria can be isolated from unused paper towels (Gendron, 2012). In addition, aluminum foil was found to sustain living bacteria for up to nineteen days, and *E. coli* can survive for more the two weeks (Dickgiesser, 1979) on the surface. It would have been preferable if hot dogs could have been deposited directly into our sterile collection bags, but this was not possible since we did not want to alert vendors that hot dogs were being collected for a purpose other than food consumption.

Another possible source of contamination could be the exposure of field-collected hot dogs to air. Once we obtained the hot dogs, we immediately measured the temperature using an infrared thermometer. This was done to take a rapid temperature and to not physically touch the hot dogs. During the brief time that the hot dogs are open to the environment, air contamination might have occurred. Mancinelli and Shulls (1978) found that *Staphylococcus* was the second most popular organism found in sampled air (Mancinelli, 1978, Robertson, 2013). *Staphylococcus* is a genus that also contains a few species which are potentially pathogenic to humans (Foster, n.d.). Thus, we cannot unequivocally rule out contamination during this part of the field sampling process.

We are confident in the identification of our bacterial unknowns by the colony PCR process. Prior to starting this project we performed a double blind control using 8 colonies (of 8 different, known species) and were able to correctly identify each based on the sequence of the 16S rDNA gene using the 27F and 1492R primer set (data not shown). However, our automated Sanger dideoxy sequencing reactions typically yielded *ca.* 700 readable bases from each priming site of the full-length amplicon. In addition, the very start and end of each electropherogram are sometimes difficult to interpret and

get clean nucleotide reads, reducing the effective size of usable data from the amplicon. Thus, forward and reverse reactions would just barely overlap when aligned. So, it was not possible to compare the completely aligned, full length of the 16S locus (1500 bp). Both the forward and reverse sequences were independently BLASTed against Genbank to verify the identity of the unknown colony. When there was agreement in the results, and the e value was 10^{-4} or smaller, this was taken as confirmation of identity at the species level. In cases where there was not agreement, attempts were made to assemble the full length amplicon from the forward and reverse sequences, or internal primers of the 16S locus were utilized to get better data quality on these regions. Identification of unknowns are best when sequences of all 9 variable regions are known. Relying on only a subset of variable regions, at either the 5' or the 3' end of the locus, can be problematic (Ewing, 1998).

The potential contribution of bacterial contamination from the individual vendor to his/her product can be seen in a study conducted by Burt in 2003 (Burt, 2003). Burt and colleagues observed the hygiene of mobile food vendors (N=10) in Midtown, Manhattan for 20 minutes each. What was striking about their results is that nearly every vendor violated the New York City Health Code at least once in the 20 minutes that they were being observed (Burt, 2003). This was not considered a major issue since enforcement of NYC Health Codes was rare for that period of time. Violations included: 1) contacting food with their bare hands; 2) vending with dirty hands; 3) storing food at incorrect temperatures; 4) working with no gloves. In some cases vendors were observed to touch food right after touching mucous membranes (i.e., mouth and nose) on their bodies (Burt, 2003). They conclude that these violations are not only acting against the

NYC Health Code but also "compromise the safety of the vendor prepared foods" (Burt, 2003). They attribute the "dangers of unsanitary vending" exhibited by the vendors to the lack of law enforcement (Burt, 2003). They also believe that there is no good data linking mobile food vending to food poisoning cases because the city inspections would often occur weeks to months after a consumer blames a pushcart for an illness (Burt, 2003).

In summary, we have examined both on-street hot dogs from 26 anonymous mobile vendors in Manhattan as well as 5 brands of store-bought control hot dogs for culturable bacteria. We have found that on-street hot dogs have more bacteria, both qualitatively and quantitatively, than control hot dogs. We have also found that 20% of sites sampled in New York contained bacteria that were resistant to one or more antibiotics. Our data also suggest an inverse correlation between serving temperature and CFU's of on-street hot dogs, with temperatures below the New York City Health Department standard of 145 F showing relatively higher CFU's. Approximately 9% (2/22) of the bacterial species found associated with on-street hot dogs are potentially pathogenic, while no pathogens were encountered in the controls. Although this study was a preliminary investigation of microbial diversity in on-street hot dogs, it should be reiterated that these results only identify microorganisms that are culturable under aerobic conditions at 37C. Considering the well established fact that less than 1% of all bacteria from environmental samples is culturable, we believe that numbers measured in this exploratory survey represent minimum values. A further examination employing NextGen sequencing methodologies may clarify and extend this current study by allowing an examination of the complete microbiome of this popular food.

A Note on Future Studies

During the course of this research project it became clear that the most obvious and useful extension of this project was to employ NextGen sequencing technologies to more completely assess the microbiome of on-street hot dog water and controls. To that end, we have isolated total DNA from all sites 26 field sites and 5 controls. DNA samples were pooled into experimental and control samples in triplicate. MetaVx NGS analysis of the prokaryotic 16S locus was performed by GeneWiz, Inc. (South Plainfield, NJ). All raw and processed data generated by GeneWiz is appended to this thesis (Appendix D). Since the inclusion and analysis of the NGS data was beyond the original proposed scope of this thesis, it is included here without analysis.

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Appendix A. PCR Reactions

Single Reaction (total volume 20 µl)

Volume (µl)	Reagent	Final Concentration
10.0	Choice Taq Mastermix* (2X stock. Contains *Taq* DNA polymerase, dNTPs, $MgCl_2$, buffer)	1X
1.0	Forward Primer (10 pmoles/µl)	0.5 µM
1.0	Reverse Primer (10 pmoles/µl)	0.5 µM
1.0	template DNA	
7.0	Sterile d.i. water	
20.0 µl	total rxn. volume	

***2X ChoiceTaq Mastermix**: 2.5U of DNA polymerase, 20 mM Tris-HCl (pH 9.0), 3 mM $MgCl_2$, 20 mM KCl, 16 mM $(NH_4)_2SO_4$, 0.1% (v/v) NP-40, 1.6 mM dNTP mix (0.4 mM ea.). Buffer produces a final Mg^{2+} concentration of 1.5 mM.

Description	Temperature	Time
Initial Denaturation	94C	2 minutes
30 Cycles	94C	15 seconds
30 Cycles	53-57C	15 seconds
30 Cycles	68C	60 seconds per kb
Final Extension	68C	5 minutes
Hold	4C	infinite time

Table 9. PCR Cycle

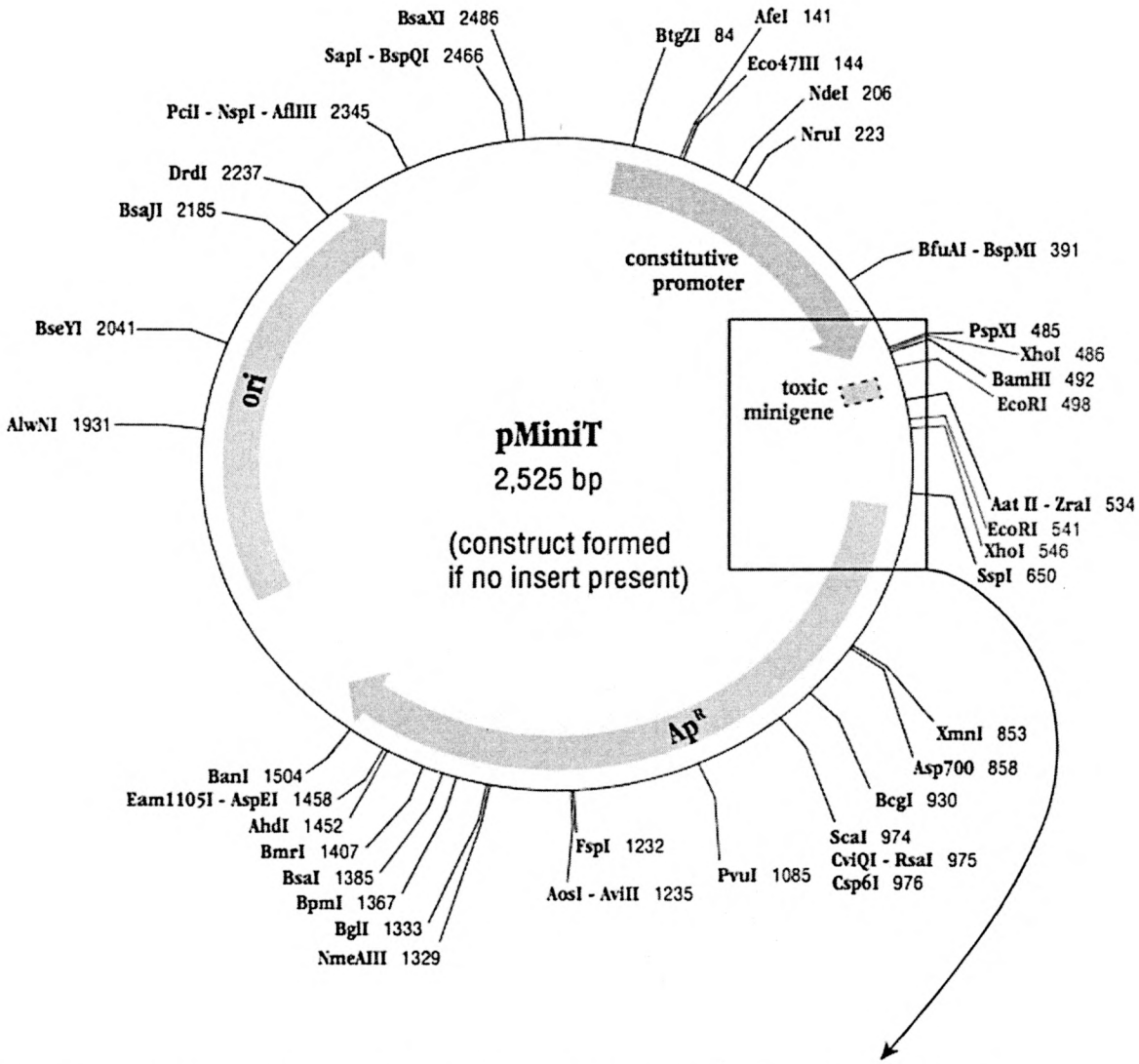
Master PCR Mix (calculated for 25 20 µl rxns).

Volume (µl)	Reagent	Final Concentration
250.0	Choice Taq Mastermix* (2X stock. Contains *Taq* DNA polymerase, dNTPs, $MgCl_2$, buffer)	1X
25.0	Forward Primer (10 pmoles/µl)	0.5 µM
25.0	Reverse Primer (10 pmoles/µl)	0.5 µM
---	template DNA	
175.0	Sterile d.i. water	
475.0 µl	total rxn. volume	

NB - Everything has been added to the master mix (MM) except for the template DNA. Take 19 µl of the MM and add 1 µl of your template DNA, mix, spin briefly and you're ready to amplify. Choice Taq Mastermix is from Denville Scientific (Denville, NJ).

***2X ChoiceTaq Mastermix**: 2.5U of DNA polymerase, 20 mM Tris-HCl (pH 9.0), 3 mM $MgCl_2$, 20 mM KCl, 16 mM $(NH_4)_2SO_4$, 0.1% (v/v) NP-40, 1.6 mM dNTP mix (0.4 mM ea.). Buffer produces a final Mg^{2+} concentration of 1.5 mM.

Appendix B. pMiniT



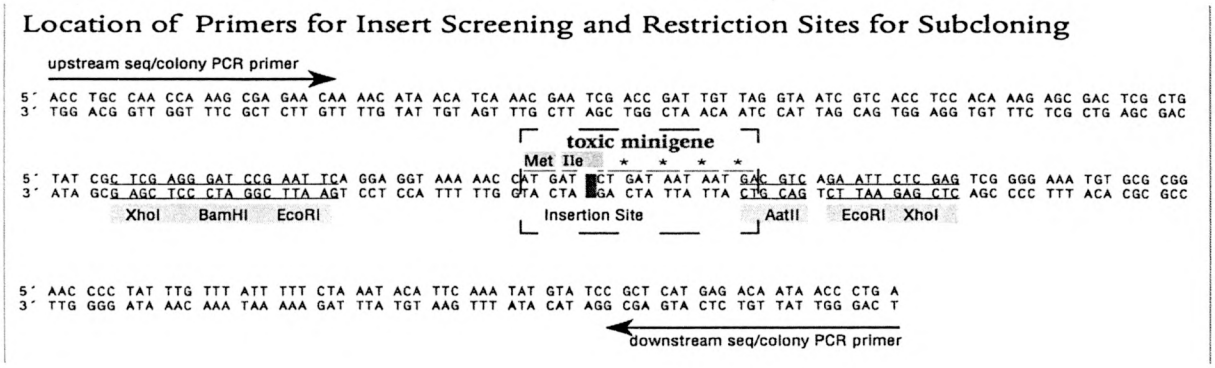


Figure 10. pMiniT

Name of Sequences	Sequences 5' to 3'
pMiniT Forward Primer	ACCTGCCAACCAAAGCGAGAAC
pMiniT Reverse Primer	TCAGGGTTATTGTCTCATGAGCG

Table 10. pMiniT Primers

Appendix C. NextGen Prep

MetaVx™ 16S rDNA Sequencing Library Preparation and Illumina MiSeq Sequencing technique

1. They first put the samples through a Qubit 2.0 Fluorometer and a 0.6% agarose gel to verify the quantity of DNA that we had in the Eppendorf tube.
2. Then they used the MetaVx 16S rDNA Sequencing Library Preparation kit to make a sequencing library of the samples.
3. They then made amplicons using 50 nanograms (ng) of the sample DNA, these amplicons were used to cover 16S rDNA V3, V4 and V5 hypervariable regions (of bacteria and archaea).
4. The amplicons also contain a short adapter added to their ends.
5. The Agilent 2100 Bioanalyzer was used for sequencing library and the quantities were found using a Qubit and/or a real time PCR machine.
6. The sequencing libraries are placed into the Illumina MiSeq.
7. The MiSeq Control Software (MCS) on a MiSeq instrument is used to identify bases and that is performed on a 2x250 pair-end configuration.
8. Finally the Illumina Basespace will be used to identify the taxonomy of the sequences.

Appendix D. Next Generation Sequencing of Hot Dog Water Microbiome

A. QC of Metagenomic Samples

DNA samples were processed and pooled for metagenomic analysis as described in Materials & Methods (Section H). A total of six samples were processed for NextGen sequencing, 3 experimental (E1, E2, and E3) and 3 control (C1, C2, and C3). GeneWiz recoded these samples to JG-01 through JG-06. A quality control analysis showed all samples to meet or exceed the requirements for NGS library production. The results of the QC analysis by Genewiz can be seen in Table 7 and Figure 10 below. NanoDrop 2000 and Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) analysis of DNA samples is provided in Table 7. Both DNA concentrations and A260/A280 ratios were determined for all samples. All A260/A280 ratios were *ca.* 1.8. For those samples with DNA concentrations below 80 ng/μL, they were subject to concentration in a Speed-Vac prior to library production.

Agarose gel electrophoresis of all DNA samples revealed a smear of fragment sizes ranging from *ca.* 1.5 to 6 kb (Figure 10). This is not unexpected since genomic DNA would be subject to random mechanical shearing during the isolation process. The lower molecular weight band may be residual RNA, although RNA should have been eliminated during the extraction process. This band may also represent very small fragments of DNA.

Sample/Name	GENEWIZ ID	Sample Vol. (ul)	Nanodrop 2000						Qubit			
			Dilution Factor	Nucleic Acid Conc. (ng/ul)	Actual Nucleic Acid Conc. (ng/ul)	Total Amount (ng)	A260/A280	A260/A230	Dilution Factor	Nucleic Acid Conc. (ng/ul)	Actual Nucleic Acid Conc. (ng/ul)	Total Amount (ng)
E1	JG01-E1	135	1	82.6	82.6	11151	1.81	0.67	1	24.2	24.2	3267
E2	JG02-E2	127	1	88.1	88.1	11188.7	1.79	0.67	1	25.4	25.4	3225.8
E3	JG03-E3	47	1	102	102	4794	1.78	0.7	1	26	26	1222
C1	JG04-C1	131	1	19.7	19.7	2580.7	1.89	0.36	1	8.98	8.98	1176.38
C2	JG05-C2	132	1	22	22	2904	1.84	0.38	1	9.16	9.16	1209.12
C3	JG06-C3	132	1	20.6	20.6	2719.2	1.82	0.36	1	9.36	9.36	1235.52

Table 11: NanoDrop 2000 and Qubit 2.0 Fluorometer Analyses of DNA Metagenomic Samples. All A260/A280 ratios demonstrated good purity of DNA samples. Those DNA samples that fell below 80 ng/μL we subject to concentration in a Speed-Vac.

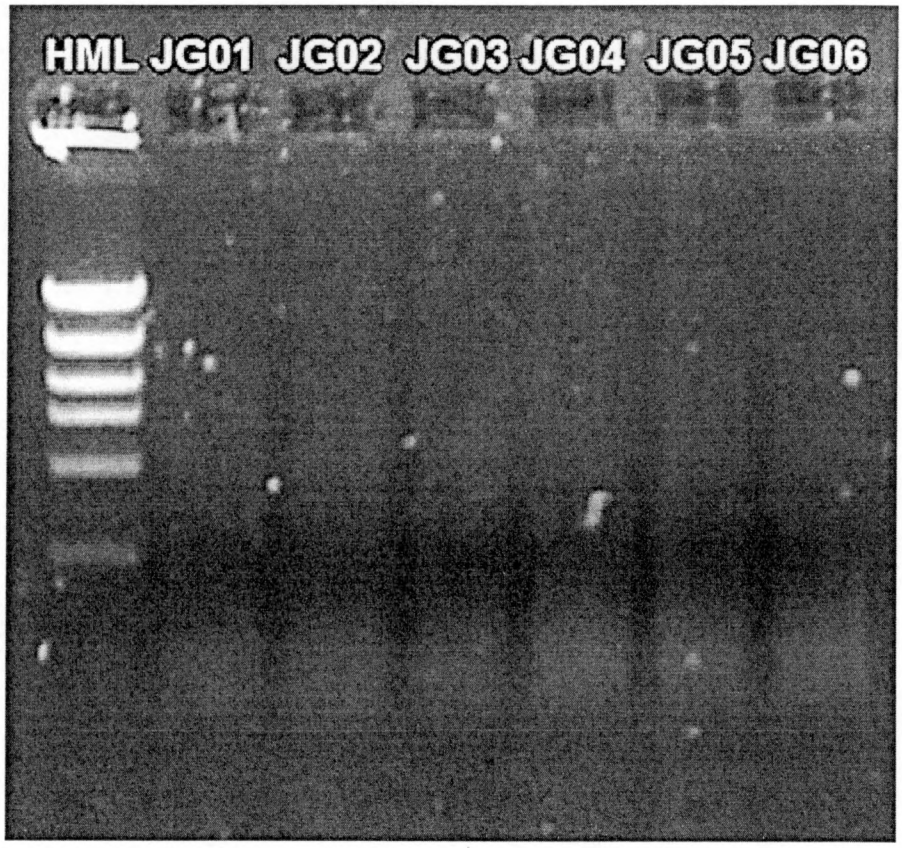


Figure 11. HML = High Mass Ladder (Invitrogen; size standards from top to bottom are 10, 6, 4, 3, 2, and 1 kb of DNA standard). Lanes JG01 through JG06 represent our metagenomic DNA samples isolated from either experimental (JG01, JG02, JG03) or control (JG04, JG05, JG06) hot dogs.

B. Results of NGS Sequencing

As can be seen in Table 8, the average number of raw reads per sample for all six samples was *ca.* 2.2 million. More importantly, we are confident in the data since in all cases the number of reads passing the quality filtering was greater than 90%.

Sample	C1		C2		C3		E1		E2		E3	
Total Reads	2,426,346		2,223,991		2,554,165		1,987,623		2,192,708		2,688,840	
	Reads Passing Quality Filtering	% Reads Passing Quality Filtering	Reads Passing Quality Filtering	% Reads Passing Quality Filtering	Reads Passing Quality Filtering	% Reads Passing Quality Filtering	Reads Passing Quality Filtering	% Reads Passing Quality Filtering	Reads Passing Quality Filtering	% Reads Passing Quality Filtering	Reads Passing Quality Filtering	% Reads Passing Quality Filtering
	2,219,977	91.5 %	2,219,977	91.5%	2,331,157	91.3%	1,816,915	91.4%	2,022,481	92.2%	2,444,648	90.9%

Table 12: Genewiz MiSeq Run Statistics for samples C1, C2, C3, E1, E2, E3.

As detailed in Materials & Methods (Section K), sequencing libraries were constructed using a MetaVx™ 16S rDNA Library Preparation kit (GENEWIZ, Inc., South Plainfield, NJ, USA). Genomic DNA (gDNA) from control and experimental samples was used to generate amplicons that cover the V3, V4 and V5 hypervariable regions of bacteria and Archaea 16S rDNA. Indexed adapters were added to the ends of the 16S rDNA amplicons by limited cycle PCR. Sequencing libraries were validated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and quantified by Qubit and real time PCR (Applied Biosystems, Carlsbad, CA, USA). DNA libraries were multiplexed and loaded onto an Illumina MiSeq instrument according to manufacturer's instructions (Illumina, San Diego, CA, USA). Sequencing was performed using a 2 x 250 paired-end (PE) configuration; image analysis and base calling was conducted by the MiSeq Control Software (MCS) on the MiSeq instrument. Taxonomy analysis was carried out on Illumina Basespace cloud computing platform.

An analysis of both control and experimental samples by taxonomic levels can be seen in Table 9. This shows the distribution of assembled 16S contigs identifiable from kingdom through species level. Similar to the "Geospatial Resolution of Human and Bacterial Diversity with City-Scale Metagenomics" article by Afshinnekoo and colleagues we too had large percentage of reads that were unidentifiable at the species level (2015). For controls, this was 15.17%; for experimental samples it was 40.93%. The volume of data generated by our NGS analysis was considerable and the summary reports for each sample can be found in the Appendix of this thesis (Appendices D: Control 1, Control 2, Control 3, Experimental 1, Experimental 2 and Experimental 3).

Sample	C1		C2		C3		E1		E2		E3	
	% Total Reads Classified to Taxonomic Level	Reads Classified to Taxonomic Level	% Total Reads Classified to Taxonomic Level	Reads Classified to Taxonomic Level	% Total Reads Classified to Taxonomic Level	Reads Classified to Taxonomic Level	% Total Reads Classified to Taxonomic Level	Reads Classified to Taxonomic Level	% Total Reads Classified to Taxonomic Level	Reads Classified to Taxonomic Level	% Total Reads Classified to Taxonomic Level	Reads Classified to Taxonomic Level
Kingdom	98.23%	2,180,760	98.23%	2,180,760	98.19%	2,289,034	99.84%	1,814,029	99.79%	2,018,206	99.90%	2,442,294
Phylum	97.82%	2,171,487	97.82%	2,171,487	97.77%	2,279,163	99.74%	1,812,197	99.66%	2,015,687	99.82%	2,440,142
Class	97.69%	2,168,632	97.69%	2,168,632	97.65%	2,276,300	99.71%	1,811,593	99.62%	2,014,861	99.79%	2,439,464
Order	97.50%	2,164,443	97.50%	2,164,443	97.45%	2,271,631	99.65%	1,810,624	99.56%	2,013,556	99.73%	2,438,127
Family	97.36%	2,161,467	97.36%	2,161,467	97.31%	2,268,559	99.56%	1,808,906	99.46%	2,011,529	99.64%	2,435,798
Genus	96.32%	2,138,310	96.32%	2,138,310	96.27%	2,244,272	93.23%	1,693,914	92.48%	1,870,302	93.72%	2,291,080
Species	85.12%	1,889,728	85.12%	1,889,728	84.25%	1,963,962	58.25%	1,058,343	61.64%	1,246,687	57.31%	1,401,071

Table 13: Analysis of NGS data for control and experimental samples broken down by taxonomic levels. Control samples averaged 84.83% +/- 0.50 and experimental samples averaged 59.07% +/- 2.28 for contigs identified at the species level. This means that we had 15.17% in controls and 40.93% in experimental samples unidentified at the species level.

The three replicates of the Experimental samples (E1, E2, and E3) were combined into a single master list. A species must occur in all three samples to be included on the master list. In other words, species identified in only one, or in two samples, were eliminated. The same was done in creating a master list for the Control groups. The experimental group (master list) contained 837 (+/- 30.6) bacterial species and the control group (master list) averaged 682 (+/- 43.0) bacterial species. This represents a 22.7% increase in species diversity in the experimental over the control group.

Control 1

Sample Information

Sample ID:	C1
Sample Name:	JG04-C1
Run Folder:	D:\Illumina\MiSeqAnalysis\7e0729e5f91147ca828ca5ee013f96f5
Taxonomy File:	gg_13_5_species_32bp.dat

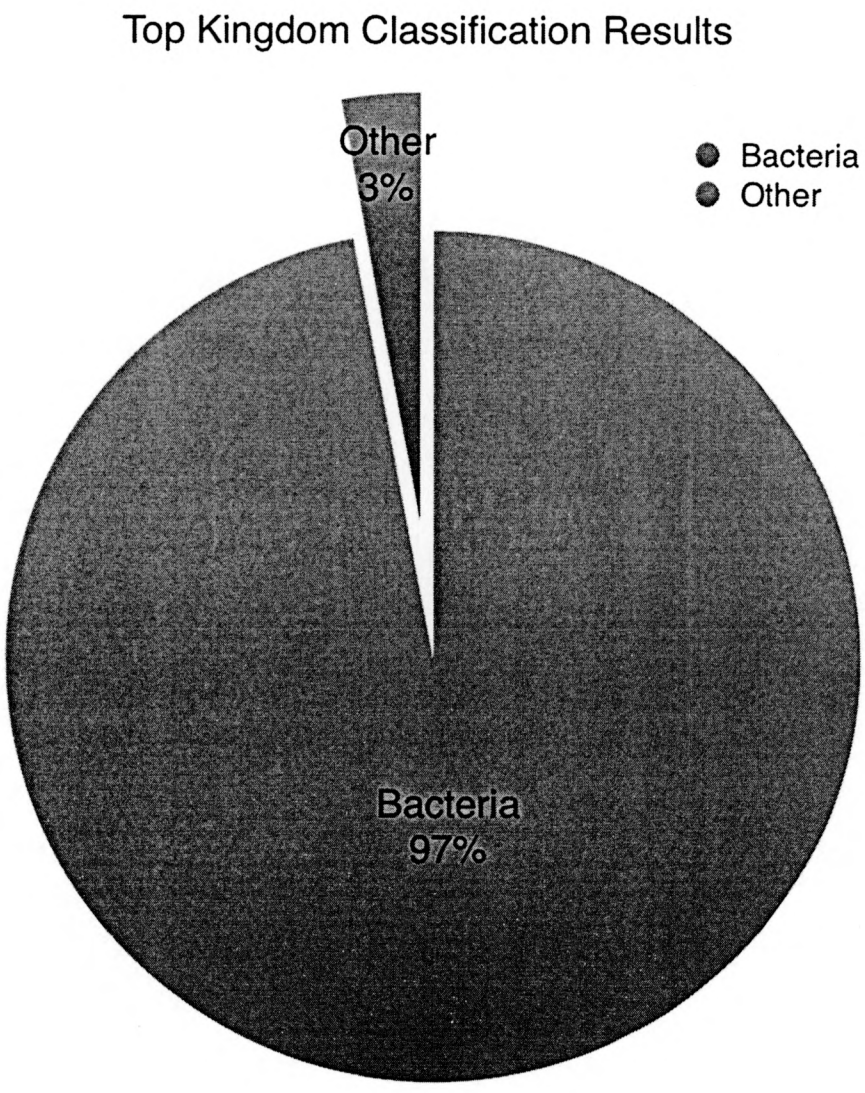
Sequencing Statistics

Total Reads	Reads Passing Quality Filtering	% Reads Passing Quality Filtering
2,426,346	2,219,977	91.5 %

The "Other" category in this pie charts are the sum of all classifications with less than 3.5% abundance.

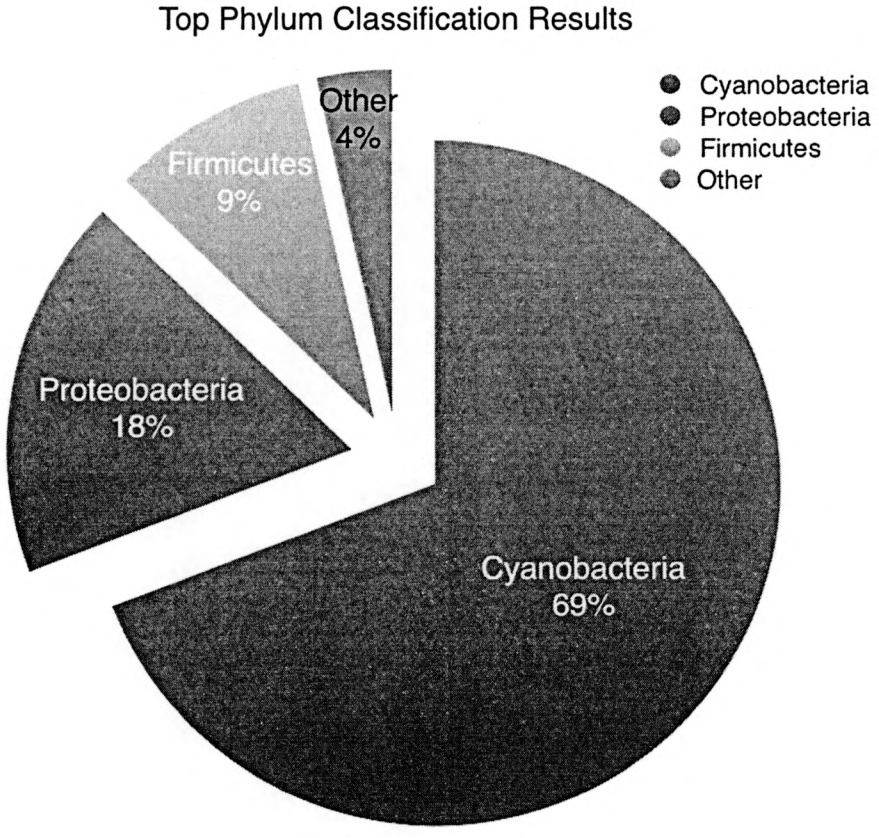
Top Kingdom Classification Results

Classification	% Total Read	Number of Read
Bacteria	97.16%	2,156,929
Unclassified at Kingdom level	1.77%	39,217
Archaea	1.07%	23,799
Viruses	0%	32



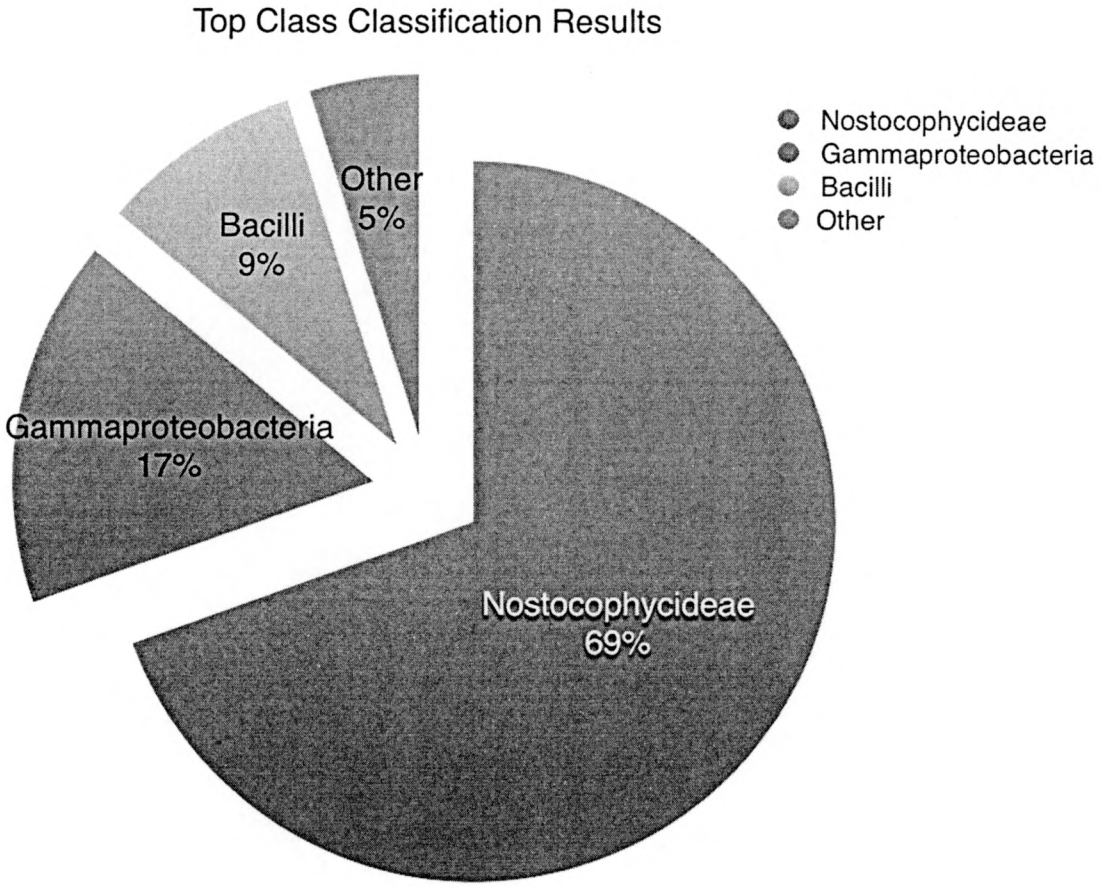
Top Phylum Classification Results

Classification	% Total Reads	Number of Reads
Cyanobacteria	69.13%	1,534,629
Proteobacteria	18.06%	399,486
Firmicutes	9.31%	206,321
Unclassified at Phylum level	2.18%	48,490
Euryarchaeota	0.85%	18,926
Crenarchaeota	0.22%	4,809
Bacteroidetes	0.17%	3,814
Actinobacteria	0.08%	1,732



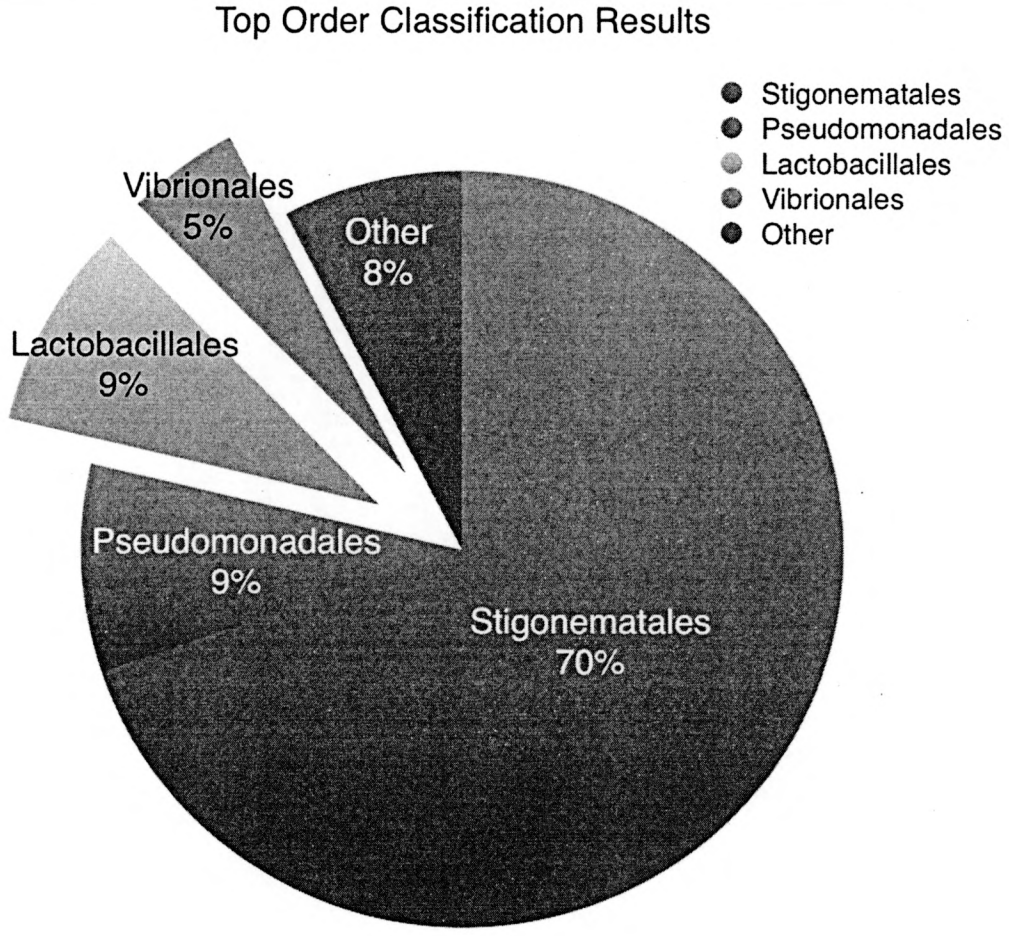
Top Class Classification Results

Classification	% Total Reads	Number of Reads
Nostocophycideae	69.44%	1,530,380
Gammaproteobacteria	16.54%	365,040
Bacilli	9.17%	199,630
Unclassified at Class level	2.31%	51,345
Alphaproteobacteria	1.12%	24,912
Methanomicrobia	0.84%	18,617
Deltaproteobacteria	0.29%	6,458
Clostridia	0.29%	6,351



Top Order Classification Results

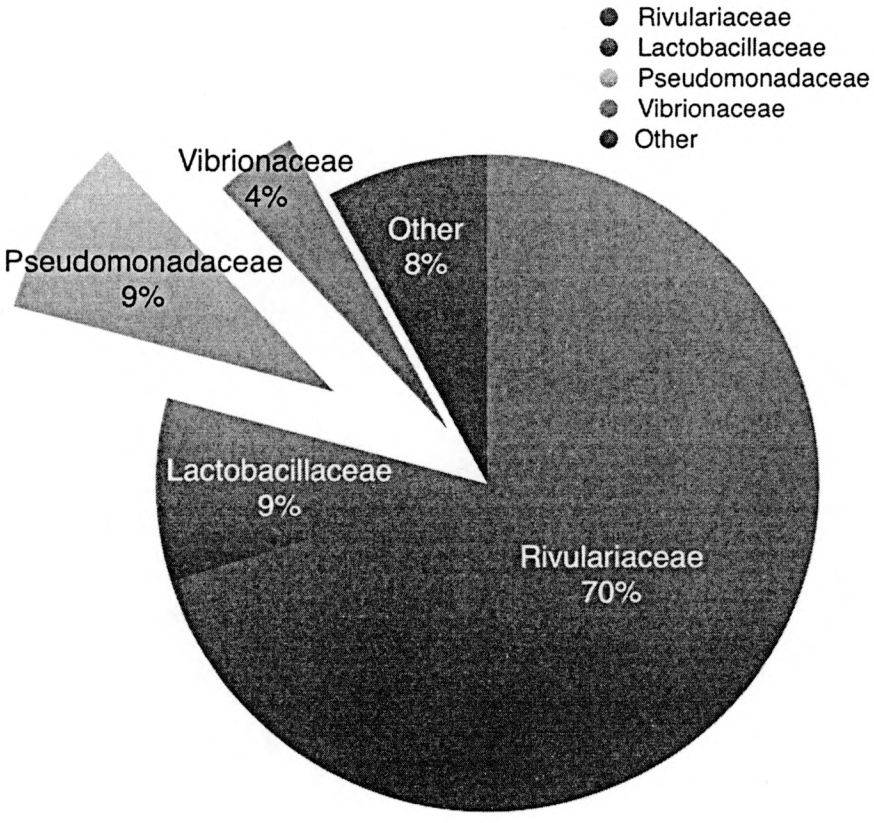
Classification	% Total Reads	Number of Reads
Stigonematales	69.75%	1,517,341
Pseudomonadales	8.91%	197,764
Lactobacillales	8.88%	197,169
Vibrionales	4.78%	87,038
Enterobacteriales	3.30%	73,157
Unclassified at Order level	2.50%	55,534
Rhodobacterales	1.04%	23,062
Methanomicrobiales	0.84%	18,601



Top Family Classification Results

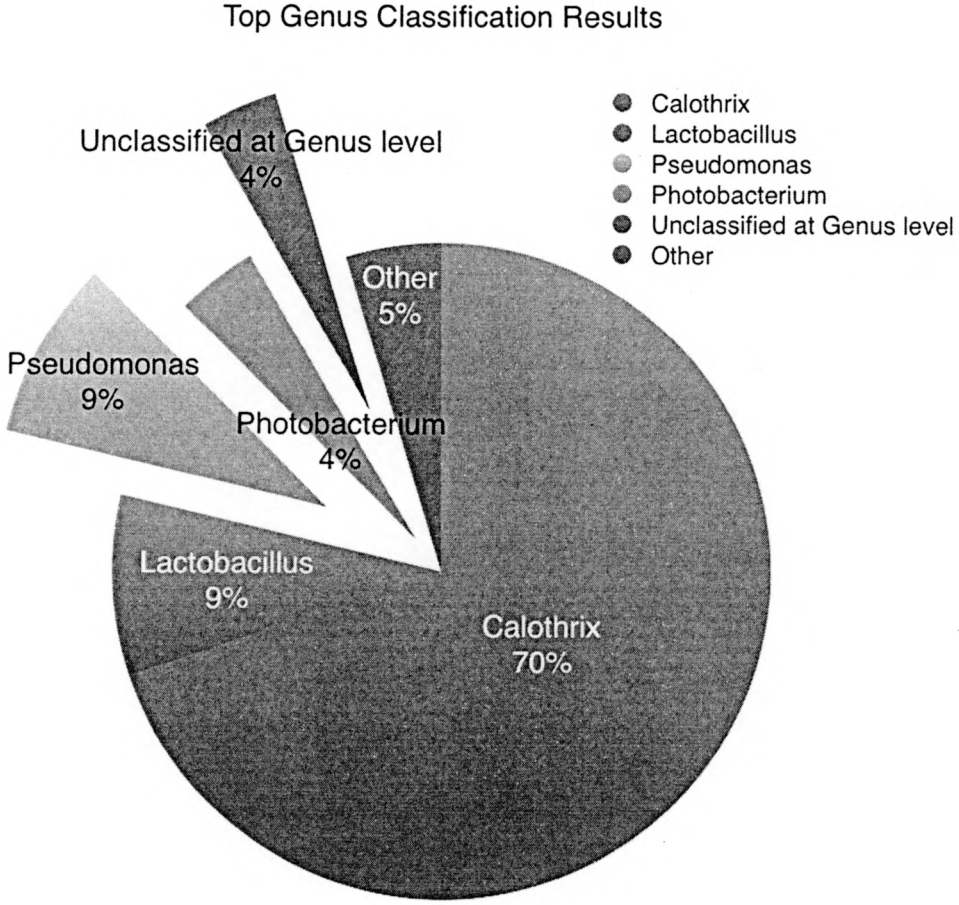
Classification	% Total Reads	Number of Reads
Rivulariaceae	70.35%	1,517,341
Lactobacillaceae	9.08%	195,169
Pseudomonadaceae	8.85%	192,057
Vibrionaceae	3.92%	87,038
Enterobacteriaceae	3.40%	73,157
Unclassified at Family level	2.64%	58,510
Rhodobacteraceae	0.92%	20,340
Methanocorpusculaceae	0.84%	18,601

Top Family Classification Results



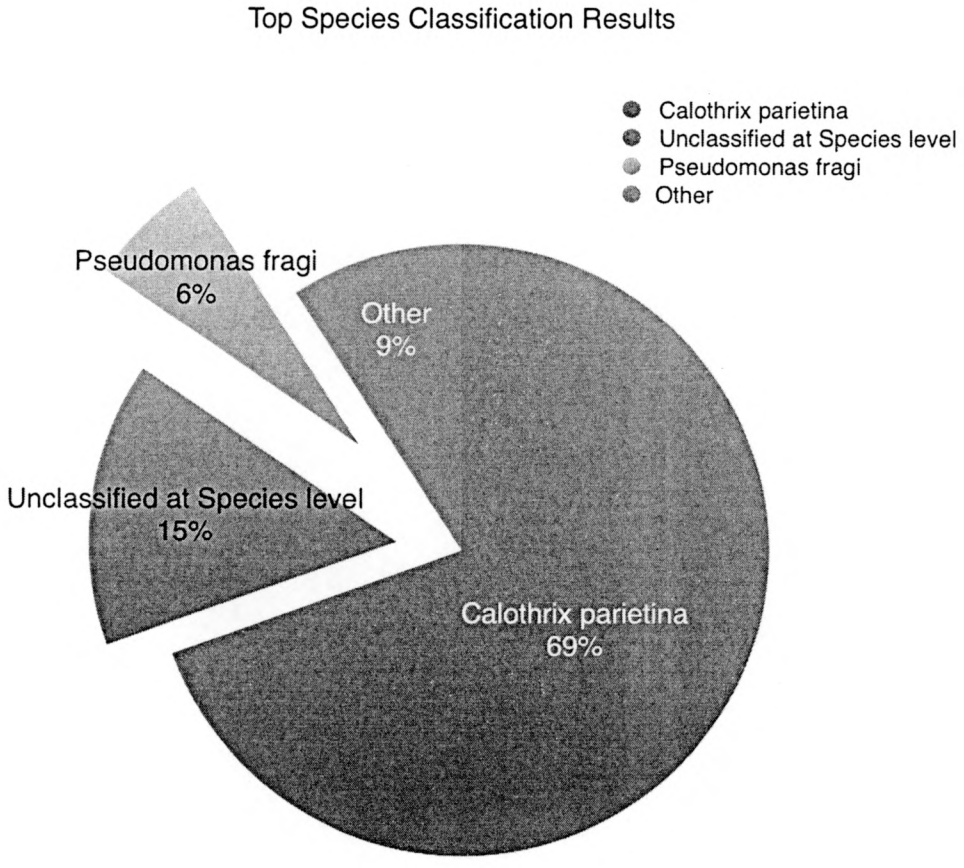
Top Genus Classification Results

Classification	% Total Reads	Number of Reads
Calothrix	70.35%	1,517,341
Lactobacillus	8.88%	192,614
Pseudomonas	8.60%	192,057
Photobacterium	3.89%	86,398
Unclassified at Genus level	3.68%	81,667
Candidatus Blochmannia	3.23%	71,773
Phaeobacter	0.91%	20,262
Nostoc	0.46%	10,282



Top Species Classification Results

Classification	% Total Reads	Number of Reads
Calothrix parietina	69.35%	1,517,328
Unclassified at Species level	14.88%	330,249
Pseudomonas fragi	6.42%	138,074
Photobacterium kishitanii	3.43%	76,233
Candidatus Blochmannia rufipes	3.42%	71,196
Pseudomonas lundensis	1.68%	37,300
Nostoc ellipsosporum	0.52%	9,332
Oscillospira guilliermondii	0.30%	4,379



Control 2

Sample Information

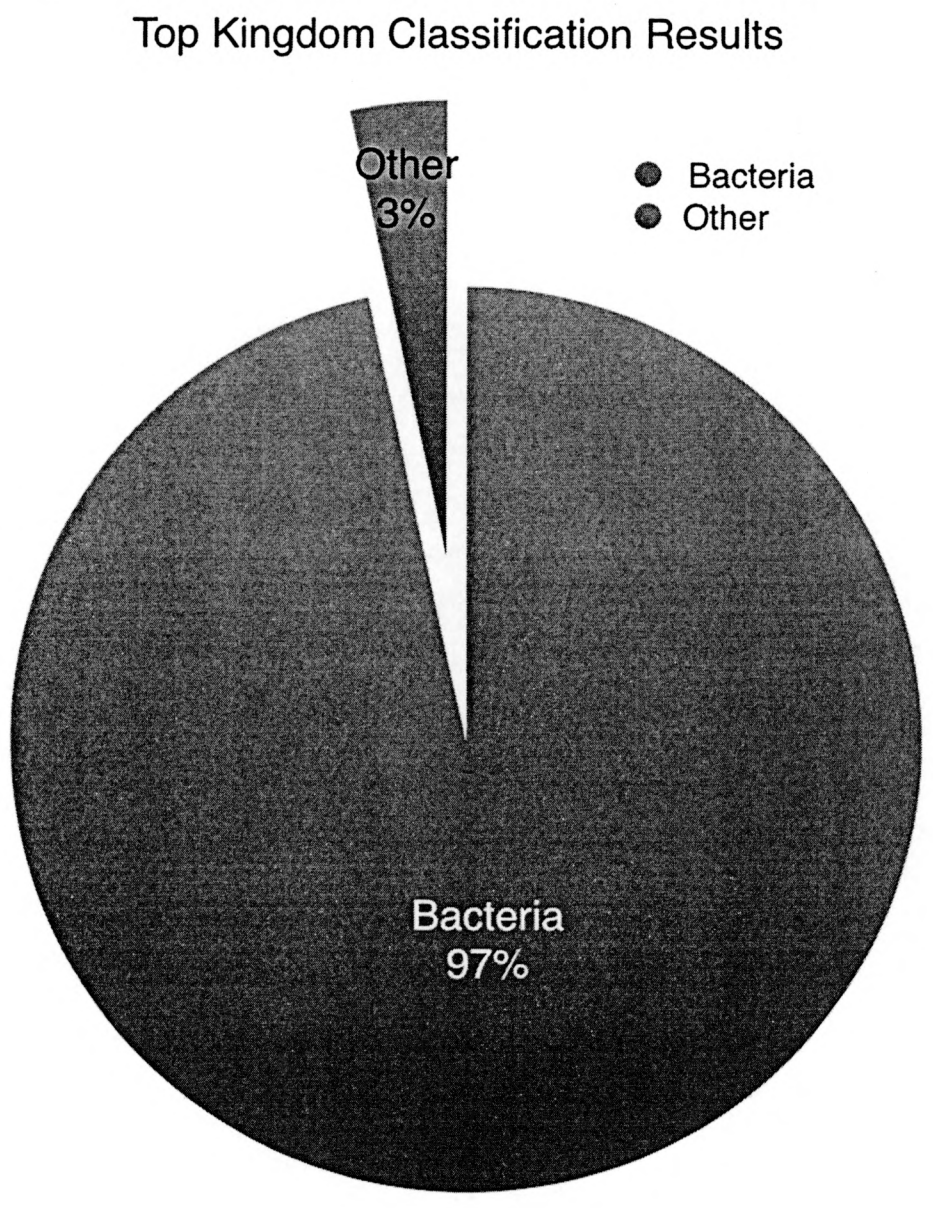
Sample ID:	C2
Sample Name:	JG05-C2
Run Folder:	D:\Illumina\MiSeqAnalysis\7e0729e5f91147ca828ca5ee013f96f5
Taxonomy File:	gg_13_5_species_32bp.dat

Sequencing Statistics

Total Reads	Reads Passing Quality Filtering	% Reads Passing Quality Filtering
2,223,991	2,034,461	91.5 %

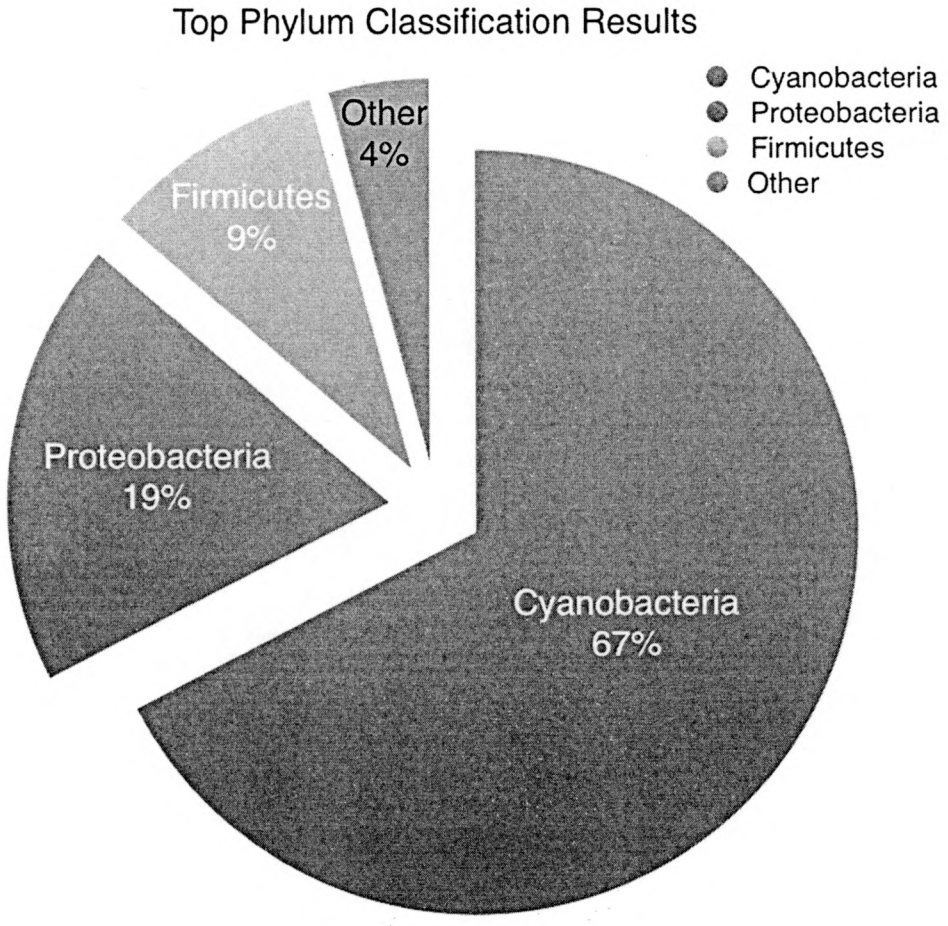
Top Kingdom Classification Results

Classification	% Total Reads	Number of Reads
Bacteria	96.54%	1,963,979
Unclassified at Kingdom level	2.40%	48,836
Archaea	1.06%	21,617
Viruses	0.00%	29



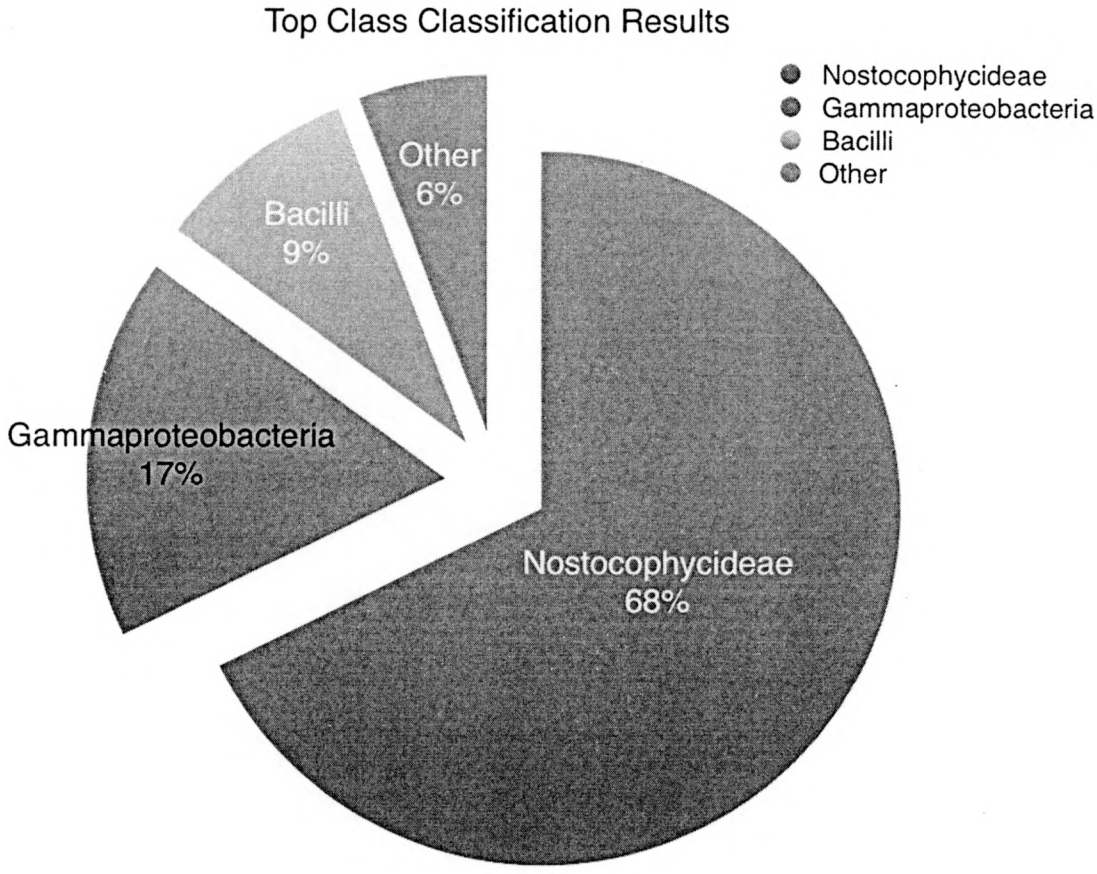
Top Phylum Classification Results

Classification	% Total Reads	Number of Reads
Cyanobacteria	67.33%	1,369,743
Proteobacteria	18.89%	384,389
Firmicutes	9.44%	191,960
Unclassified at Phylum level	2.88%	58,597
Euryarchaeota	0.85%	17,192
Crenarchaeota	0.21%	4,355
Bacteroidetes	0.21%	4,180
Actinobacteria	0.19%	1,874



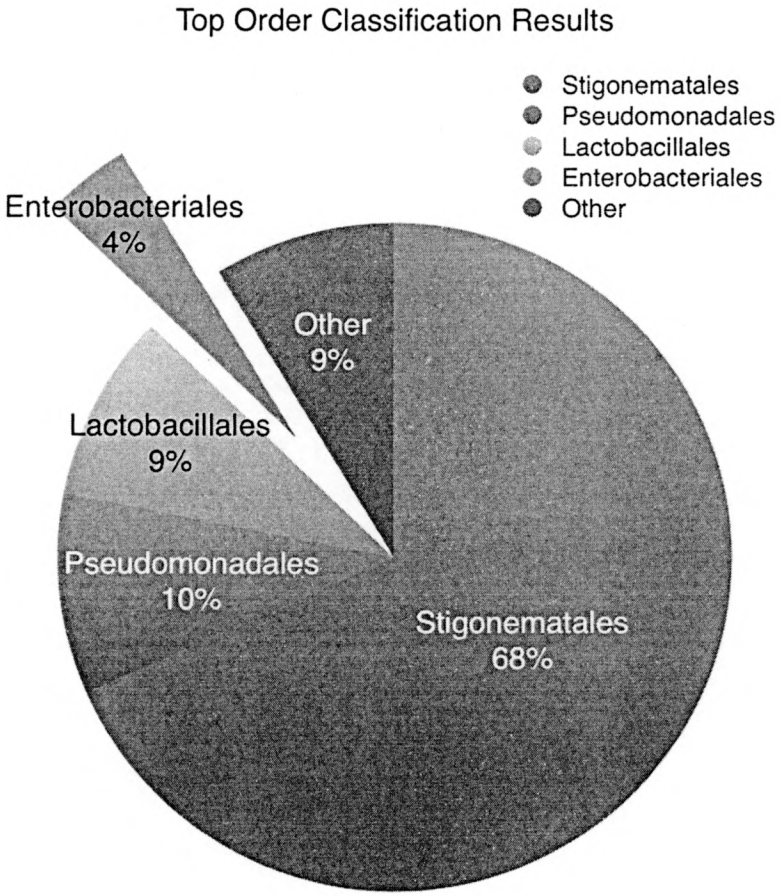
Top Class Classification Results

Classification	% Total Reads	Number of Reads
Nostocophycideae	67.91%	1,365,326
Gammaproteobacteria	17.24%	350,814
Bacilli	8.98%	182,693
Unclassified at Class level	3.12%	61,556
Alphaproteobacteria	1.14%	23,287
Methanomicrobia	0.82%	16,715
Clostridia	0.43%	8,779
Deltaproteobacteria	0.36%	7,330



Top Order Classification Results

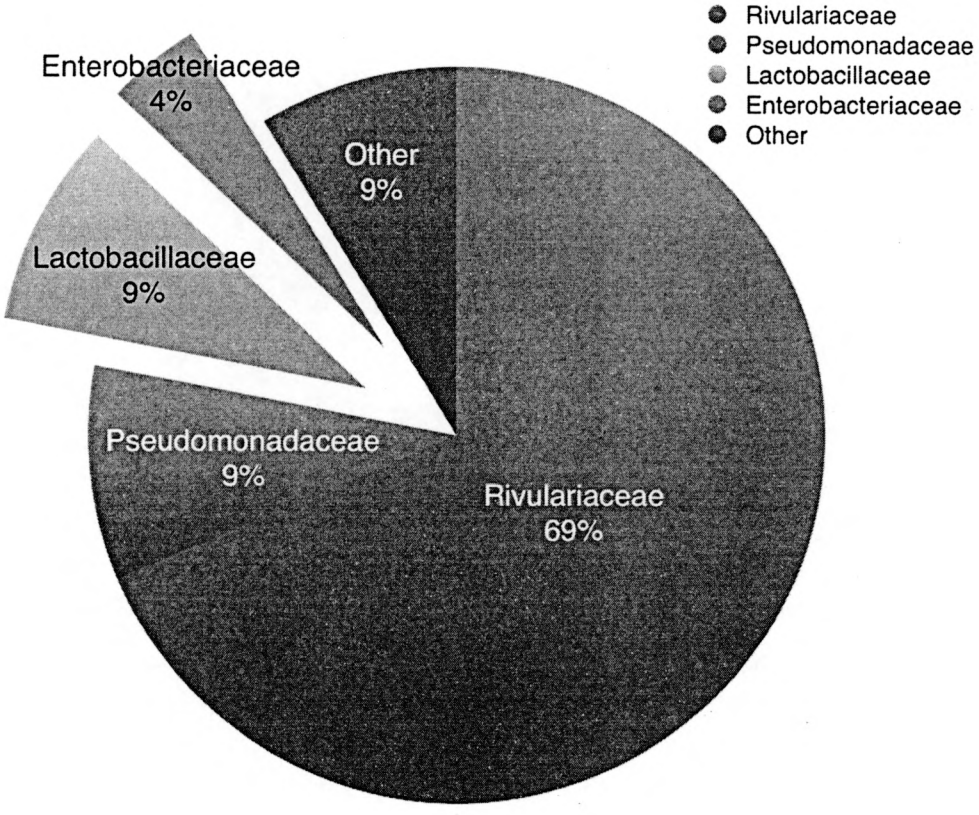
Classification	% Total Reads	Number of Reads
Stigonematales	67.96%	1,354,928
Pseudomonadales	9.55%	192,895
Lactobacillales	8.85%	180,132
Enterobacteriales	4.01%	81,554
Vibrionales	3.42%	69,505
Unclassified at Order level	3.36%	65,724
Rhodobacterales	2.03%	21,234
Methanomicrobiales	0.82%	16,699



Top Family Classification Results

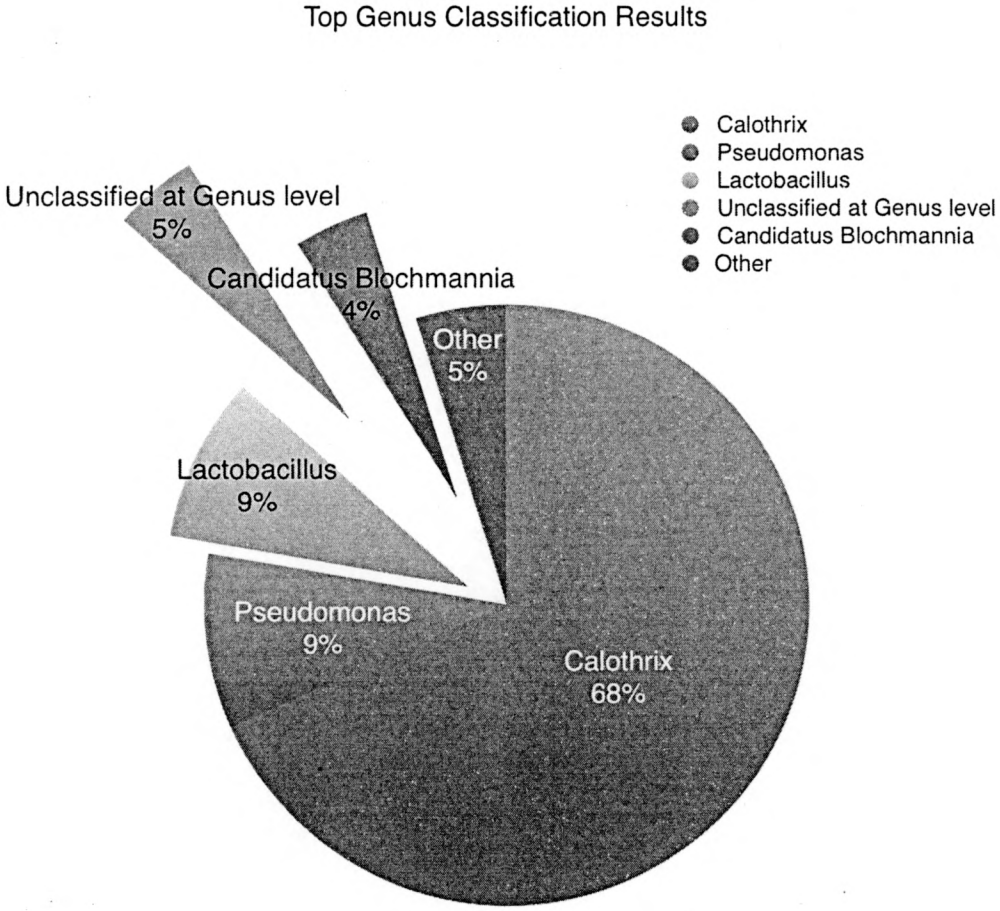
Classification	% Total Reads	Number of Reads
Rivulariaceae	68.60%	1,354,928
Pseudomonadaceae	9.49%	186,924
Lactobacillaceae	8.96%	178,186
Enterobacteriaceae	4.41%	81,554
Vibrionaceae	3.42%	69,505
Unclassified at Family level	3.37%	68,560
Rhodobacteraceae	0.93%	18,860
Methanocorpusculaceae	0.82%	16,699

Top Family Classification Results



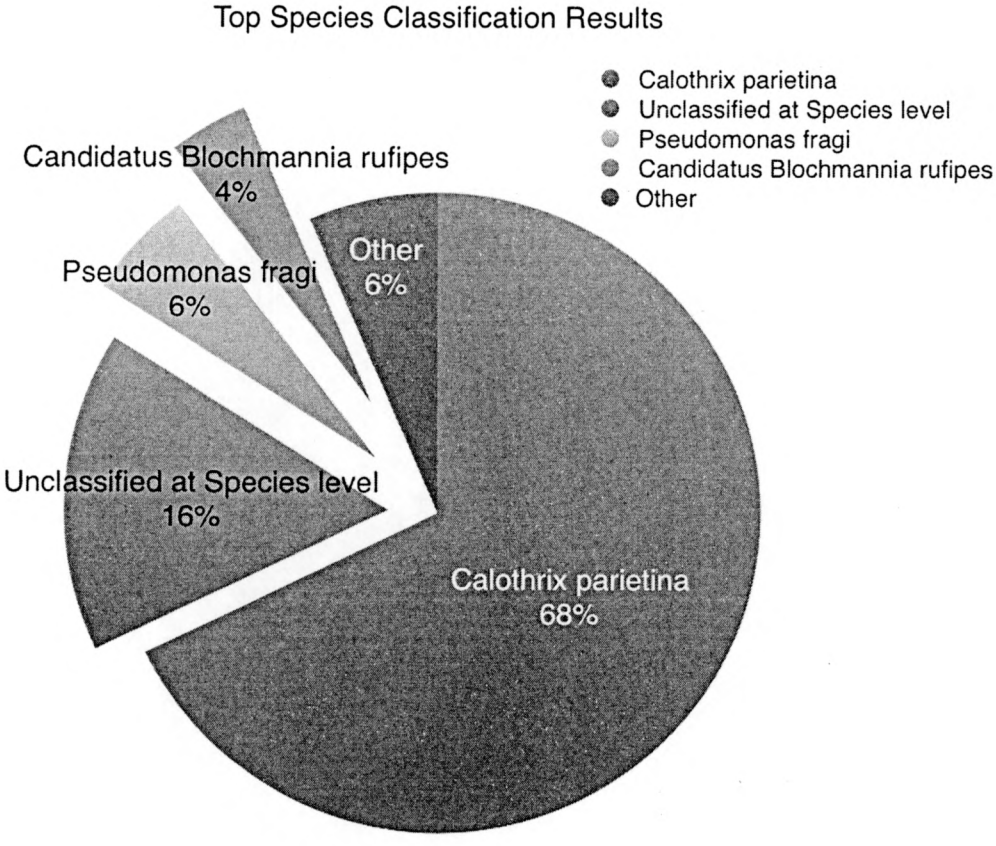
Top Genus Classification Results

Classification	% Total Reads	Number of Reads
Calothrix	68.40%	1,354,928
Pseudomonas	9.39%	186,924
Lactobacillus	8.94%	175,741
Unclassified at Genus level	4.40%	89,473
Candidatus Blochmannia	3.97%	80,096
Photobacterium	3.49%	68,946
Phaeobacter	0.92%	18,787
Nostoc	0.49%	7,958



Top Species Classification Results

Classification	% Total Reads	Number of Reads
Calothrix parietina	68.40%	1,354,917
Unclassified at Species level	15.91%	323,469
Pseudomonas fragi	5.42%	110,228
Candidatus Blochmannia rufipes	3.89%	79,183
Photobacterium kishitanii	2.85%	58,062
Pseudomonas lundensis	2.85%	57,999
Nostoc ellipsosporum	0.35%	7,060
Oscillospira guilliermondii	0.33%	6,679



Control 3

Sample Information

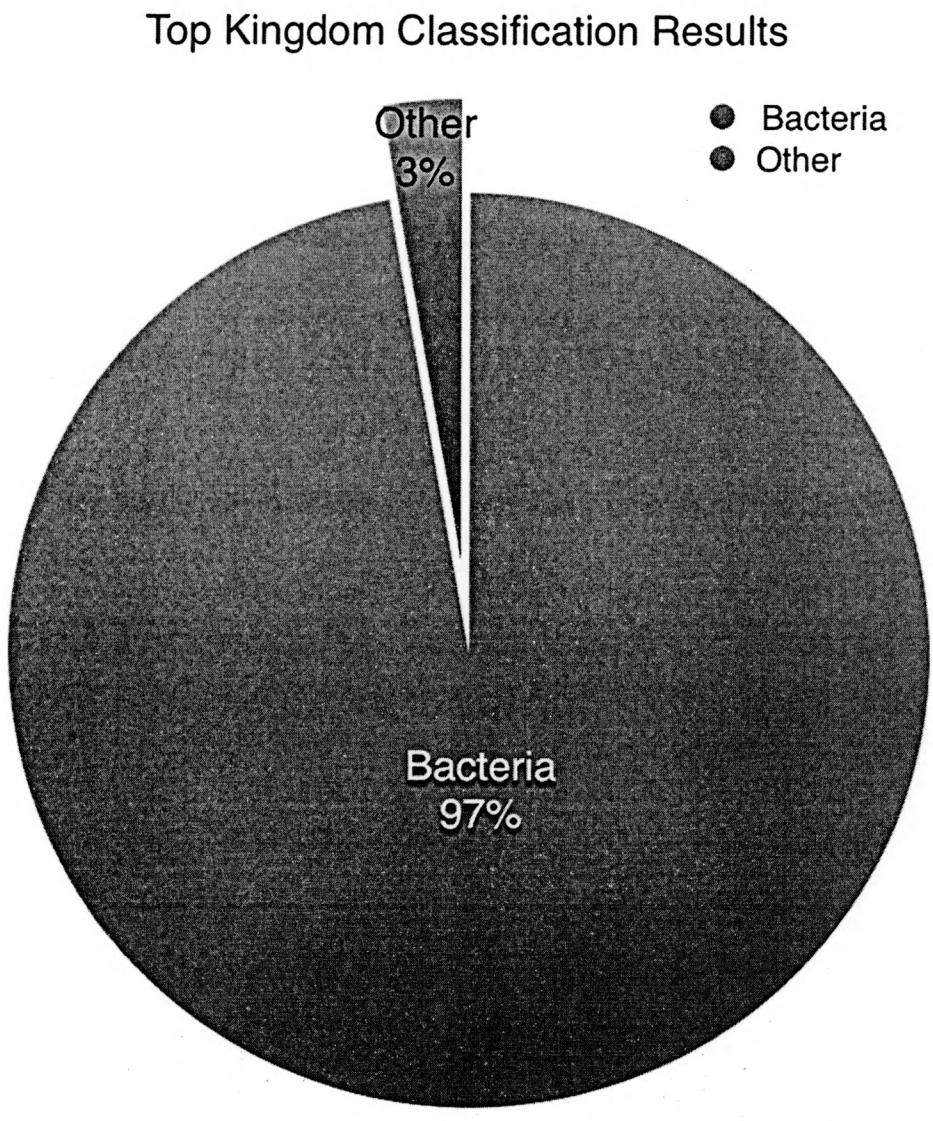
Sample ID:	C3
Sample Name:	JG06-C3
Run Folder:	D:\Illumina\MiSeqAnalysis\7e0729e5f91147ca828ca5ee013f96f5
Taxonomy File:	gg_13_5_species_32bp.dat

Sequencing Statistics

Total Reads	Reads Passing Quality Filtering	% Reads Passing Quality Filtering
2,554,165	2,331,157	91.3 %

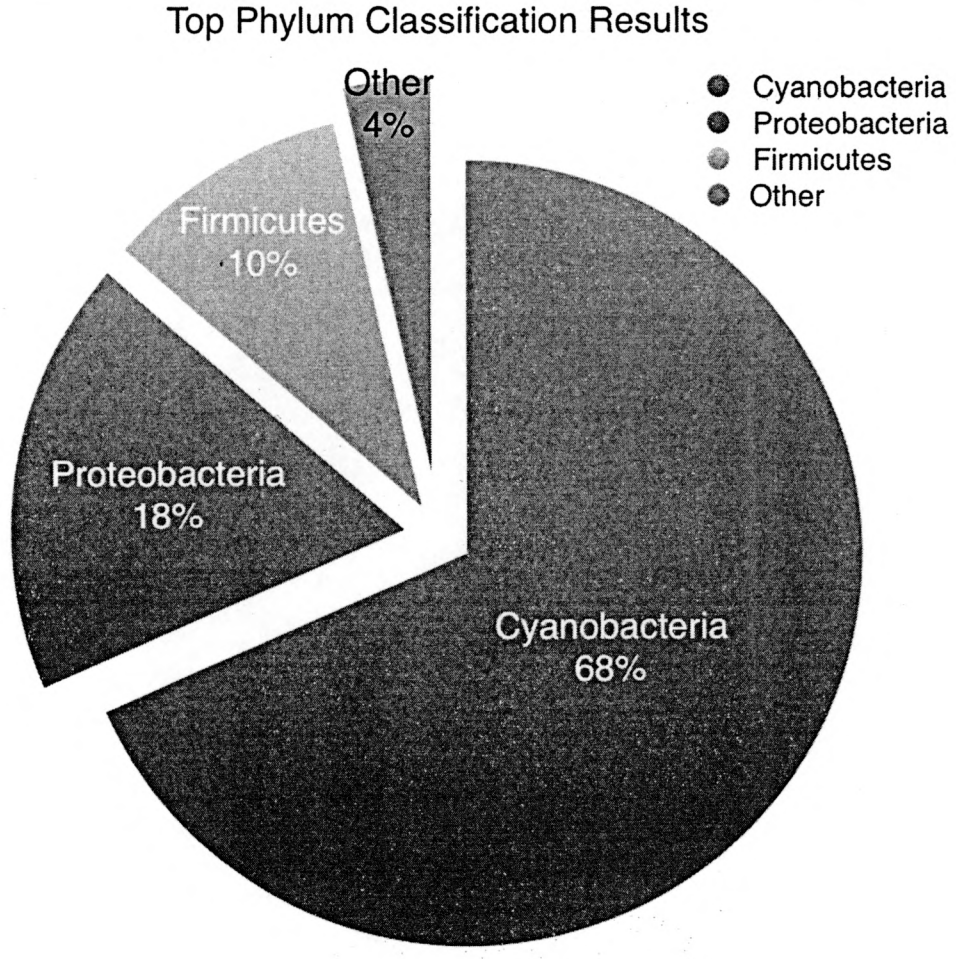
Top Kingdom Classification Results

Classification	% Total Reads	Number of Reads
Bacteria	97.11%	2,263,769
Unclassified at Kingdom level	1.81%	42,123
Archaea	1.08%	25,197
Viruses	0.00%	68



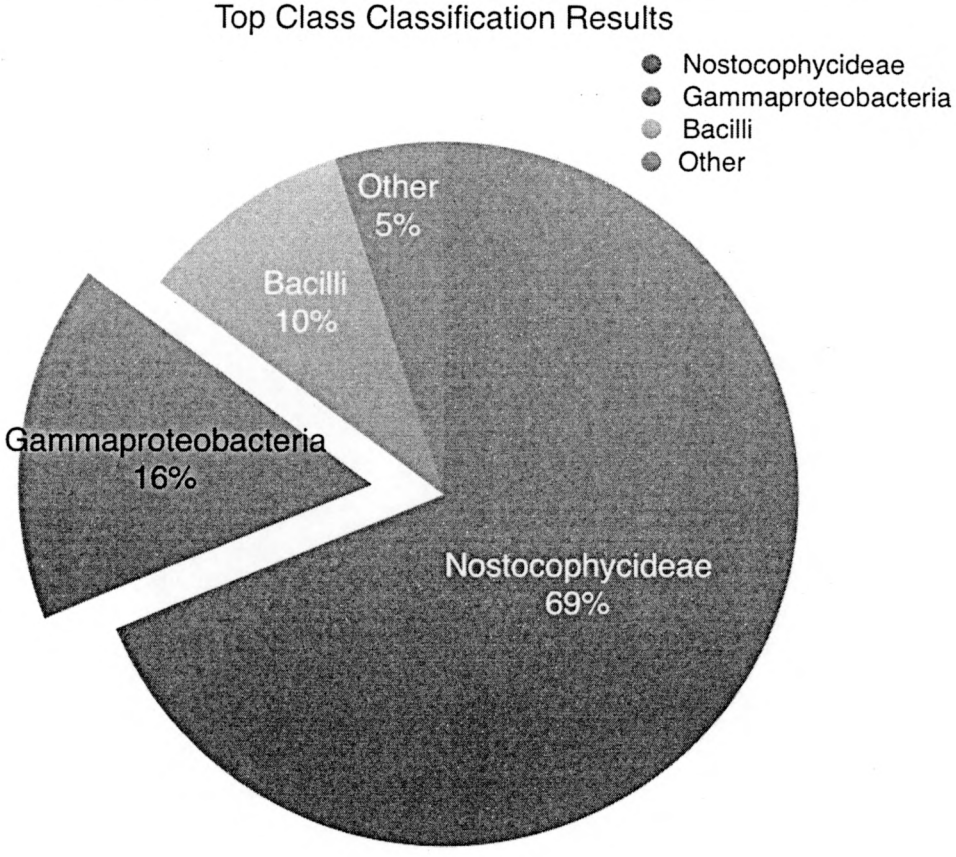
Top Phylum Classification Results

Classification	% Total Reads	Number of Reads
Cyanobacteria	68.40%	1,594,605
Proteobacteria	17.98%	419,055
Firmicutes	10.00%	233,049
Unclassified at Phylum level	2.23%	51,994
Euryarchaeota	0.86%	19,998
Crenarchaeota	0.30%	5,129
Bacteroidetes	0.15%	3,558
Actinobacteria	0.08%	1,777



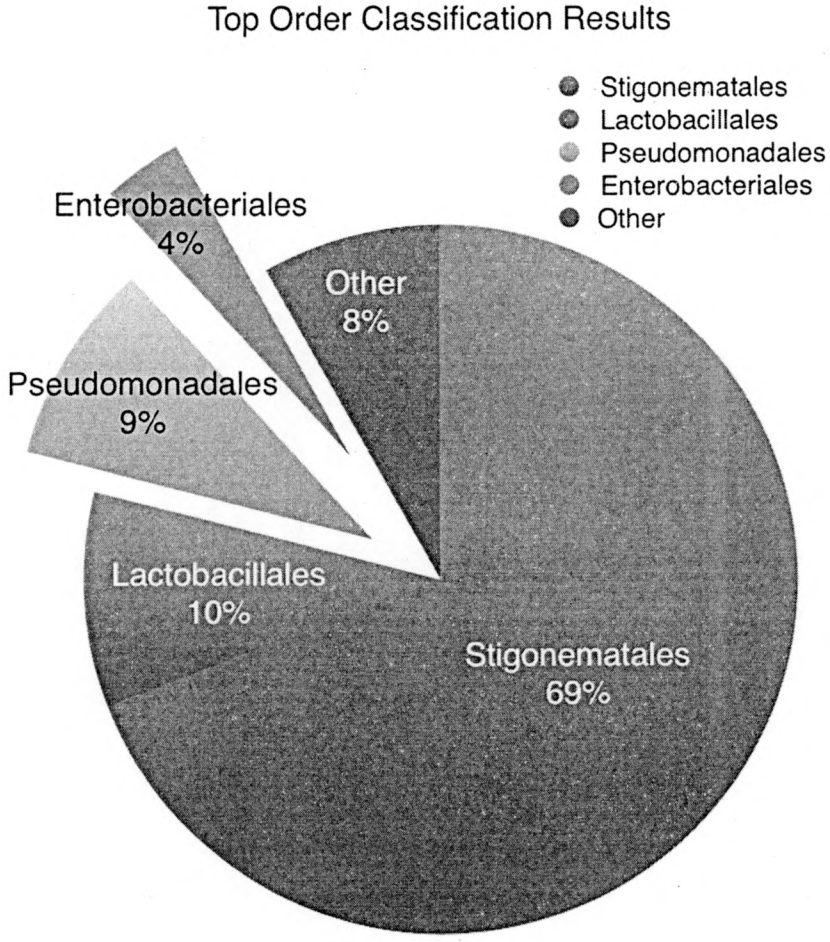
Top Class Classification Results

Classification	% Total Reads	Number of Reads
Nostocophycideae	68.91%	1,590,151
Gammaproteobacteria	16.36%	381,467
Bacilli	9.81%	226,623
Unclassified at Class level	2.35%	54,857
Alphaproteobacteria	1.16%	26,942
Methanomicrobia	0.84%	19,637
Deltaproteobacteria	0.31%	7,286
Clostridia	0.26%	6,094



Top Order Classification Results

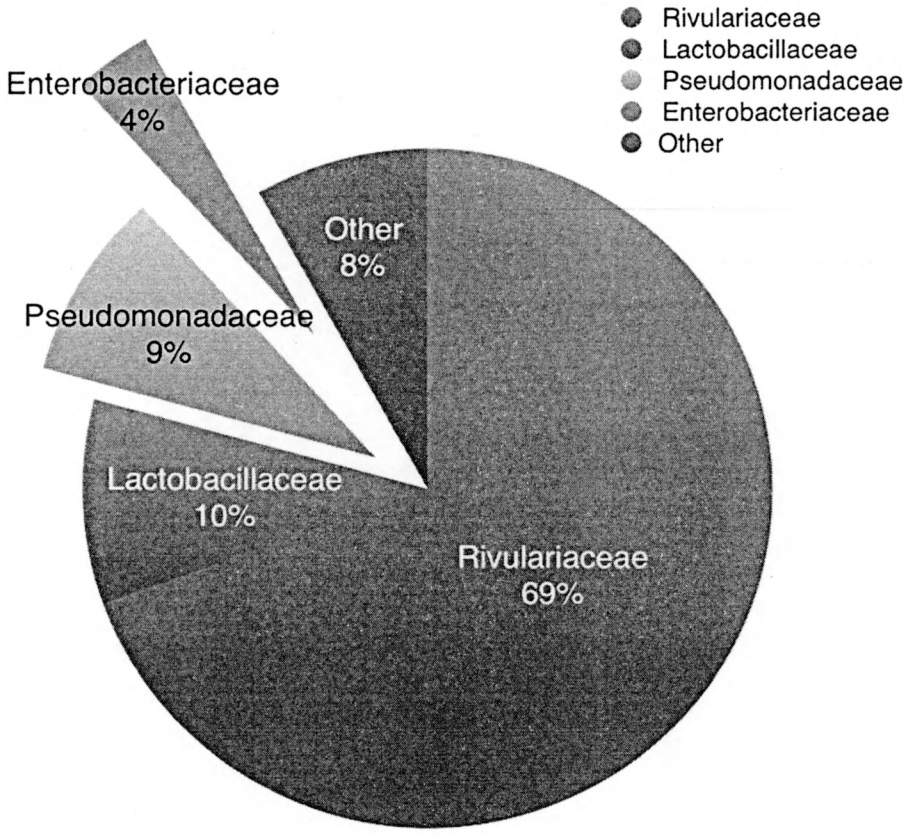
Classification	% Total Reads	Number of Reads
Stigonematales	69.43%	1,576,462
Lactobacillales	9.79%	223,621
Pseudomonadales	9.00%	209,848
Enterobacteriales	3.56%	82,970
Vibrionales	3.49%	81,302
Unclassified at Order level	2.84%	59,526
Rhodobacterales	1.05%	24,572
Methanomicrobiales	0.84%	19,627



Top Family Classification Results

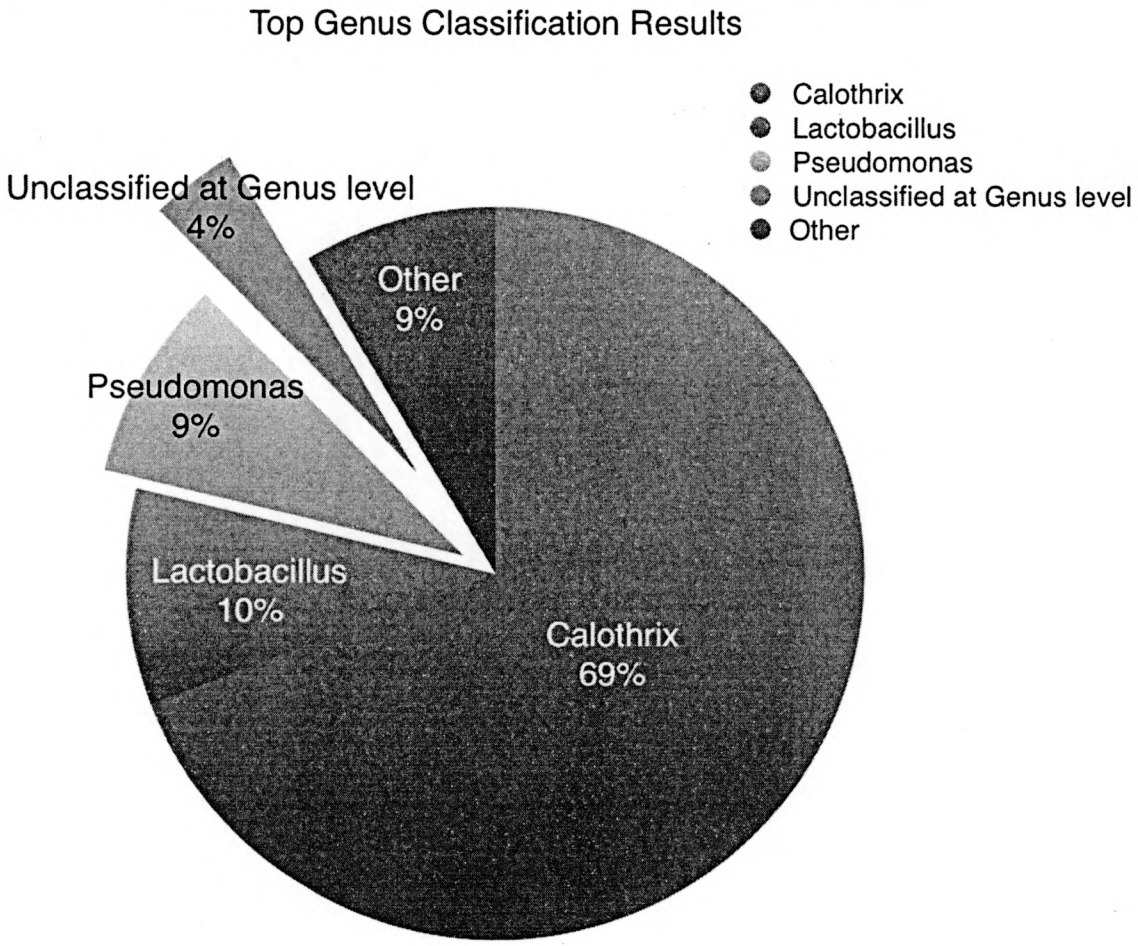
Classification	% Total Reads	Number of Reads
Rivulariaceae	69.48%	1,576,462
Lactobacillaceae	9.80%	221,441
Pseudomonadaceae	8.71%	203,045
Enterobacteriaceae	3.76%	82,970
Vibrionaceae	3.49%	81,302
Unclassified at Family level	2.99%	62,598
Rhodobacteraceae	0.93%	21,749
Methanocorpusculaceae	0.84%	19,627

Top Family Classification Results



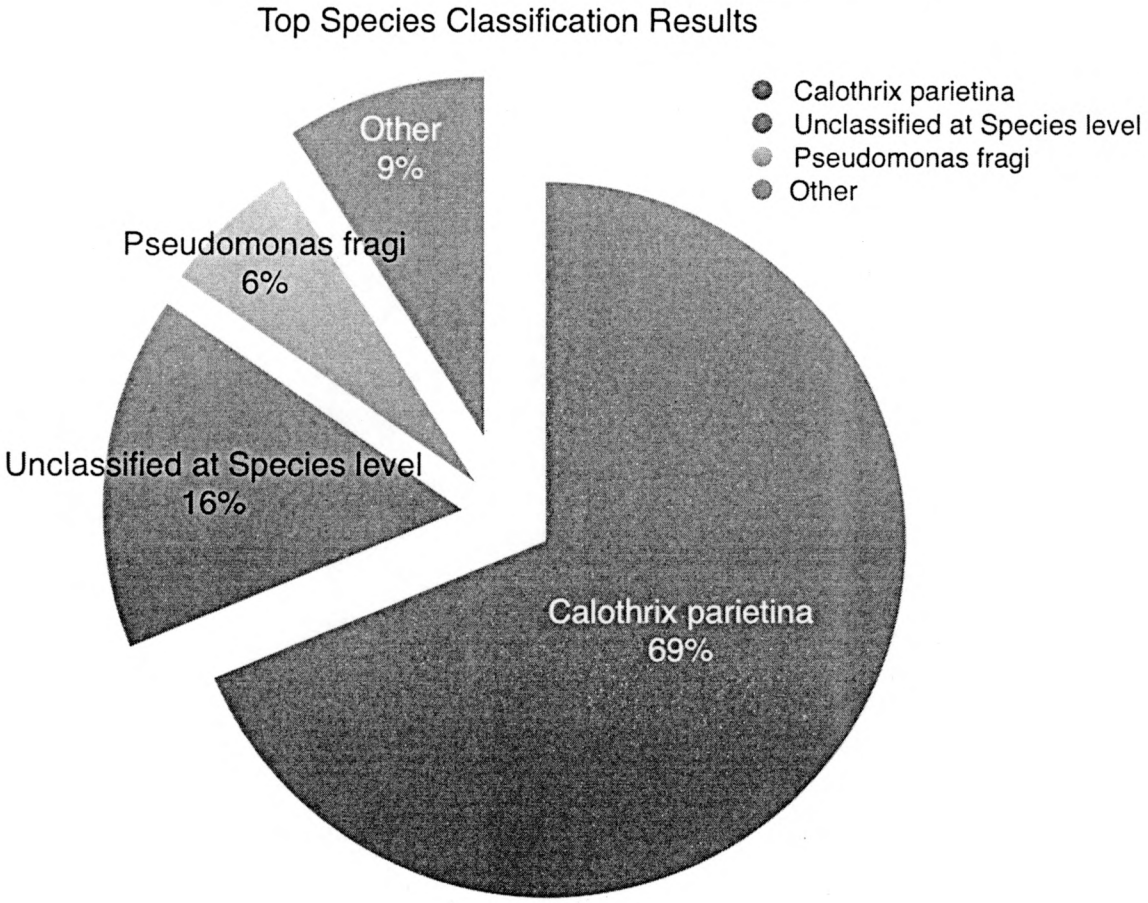
Top Genus Classification Results

Classification	% Total Reads	Number of Reads
Calothrix	69.23%	1,576,462
Lactobacillus	9.98%	218,619
Pseudomonas	8.72%	203,045
Unclassified at Genus level	3.73%	86,885
Candidatus Blochmannia	3.49%	81,311
Photobacterium	3.46%	80,689
Phaeobacter	0.93%	21,673
Nostoc	0.46%	10,637



Top Species Classification Results

Classification	% Total Reads	Number of Reads
Calothrix parietina	68.24%	1,576,440
Unclassified at Species level	16.45%	367,195
Pseudomonas fragi	6.46%	143,496
Candidatus Blochmannia rufipes	3.46%	80,561
Photobacterium kishitanii	3.04%	70,817
Pseudomonas lundensis	1.75%	40,821
Nostoc ellipsosporum	0.42%	9,765
Oscillospira guilliermondii	0.18%	4,279



Experimental 1

Sample Information

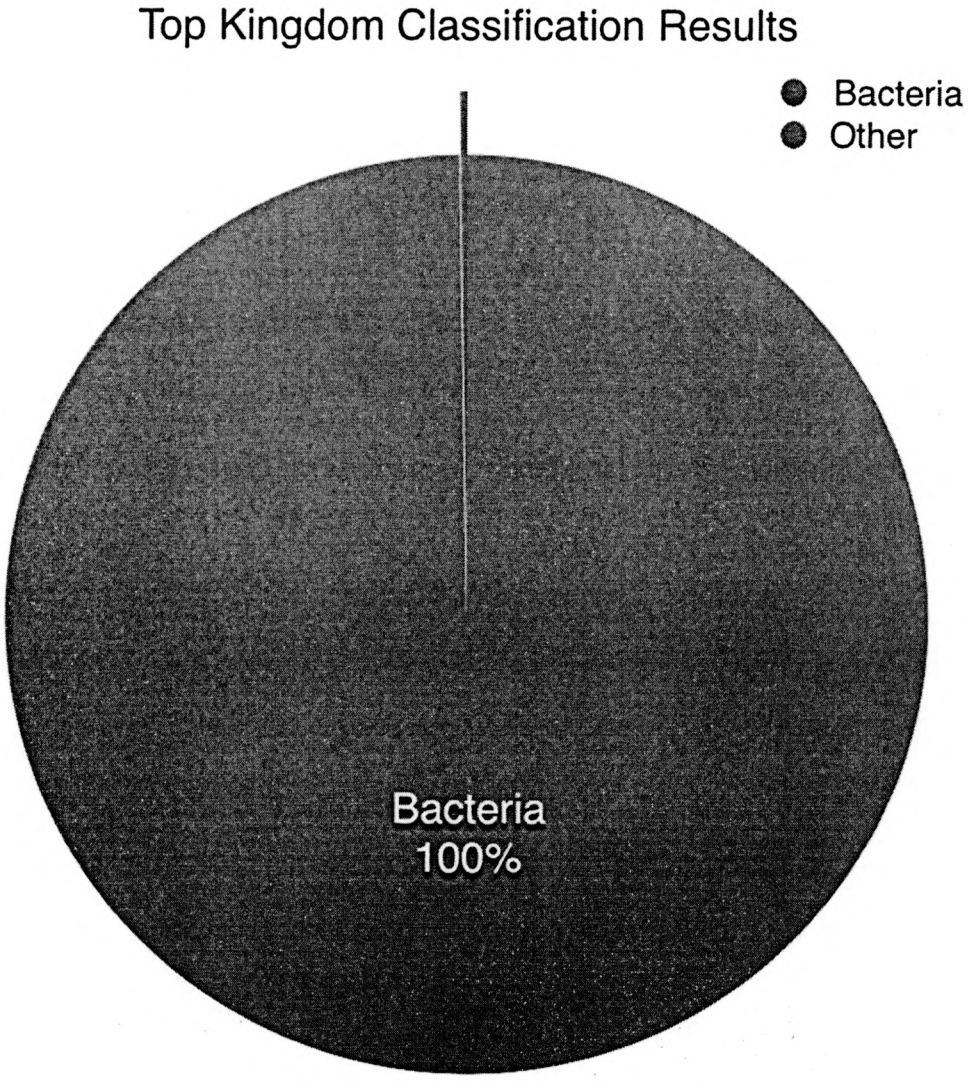
Sample ID:	E1
Sample Name:	JG01-E1
Run Folder:	D:\Illumina\MiSeqAnalysis\7e0729e5f91147ca828ca5ee013f96f5
Taxonomy File:	gg_13_5_species_32bp.dat

Sequencing Statistics

Total Reads	Reads Passing Quality Filtering	% Reads Passing Quality Filtering
1,987,623	1,816,915	91.4 %

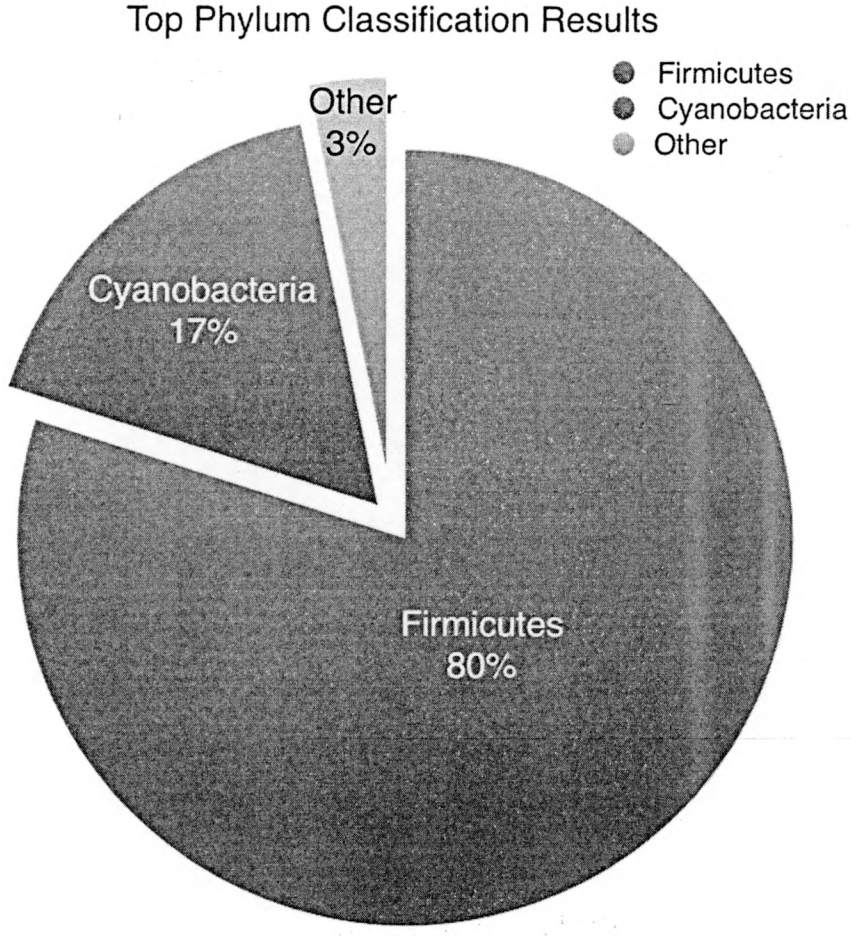
Top Kingdom Classification Results

Classification	% Total Reads	Number of Reads
Bacteria	99.72%	1,811,742
Unclassified at Kingdom level	0.16%	2,886
Archaea	0.12%	2,228
Viruses	0.00%	59



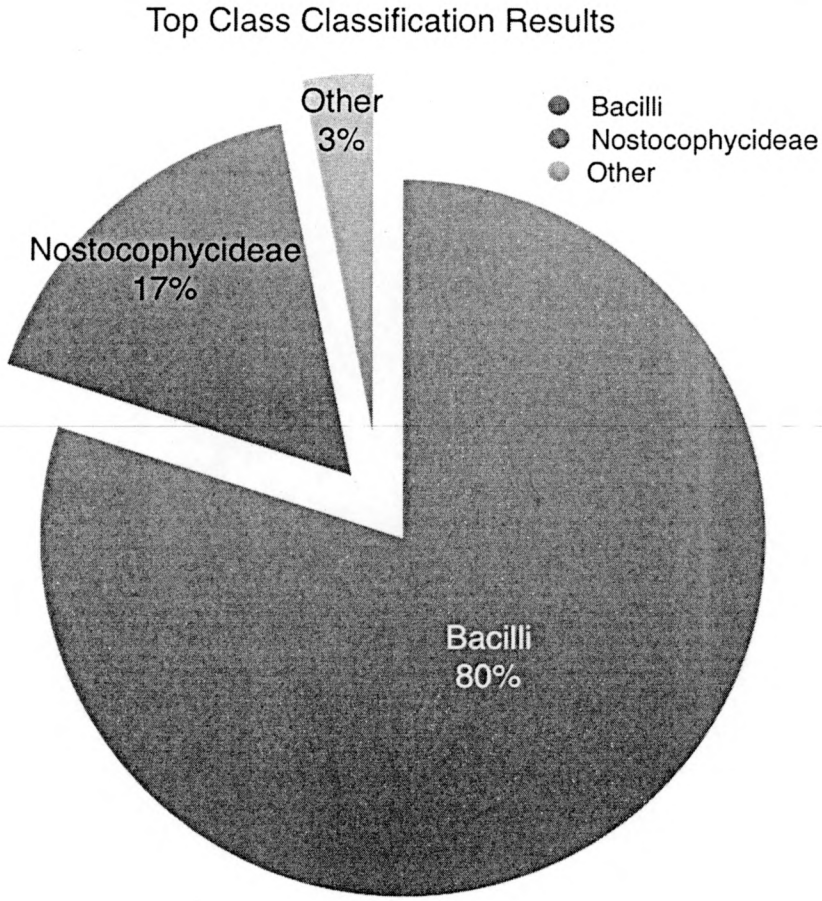
Top Phylum Classification Results

Classification	% Total Reads	Number of Reads
Firmicutes	79.95%	1,451,593
Cyanobacteria	16.85%	306,225
Proteobacteria	2.71%	49,197
Unclassified at Phylum level	0.26%	4,718
Euryarchaeota	0.10%	1,807
Bacteroidetes	0.07%	1,294
Actinobacteria	0.04%	743
Crenarchaeota	0.02%	415



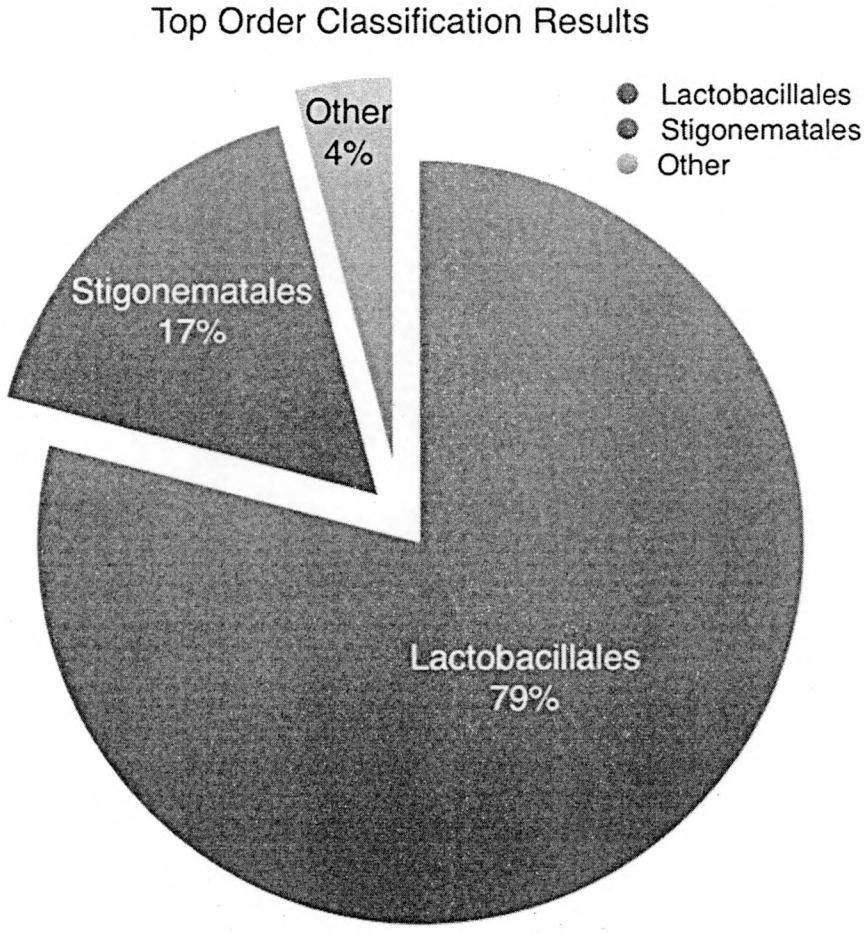
Top Class Classification Results

Classification	% Total Reads	Number of Reads
Bacilli	79.96%	1,449,917
Nostocophycideae	16.89%	305,141
Gammaproteobacteria	2.36%	42,922
Unclassified at Class level	0.29%	5,322
Alphaproteobacteria	0.28%	5,049
Methanomicrobia	0.10%	1,772
Clostridia	0.08%	1,521
Deltaproteobacteria	0.04%	793



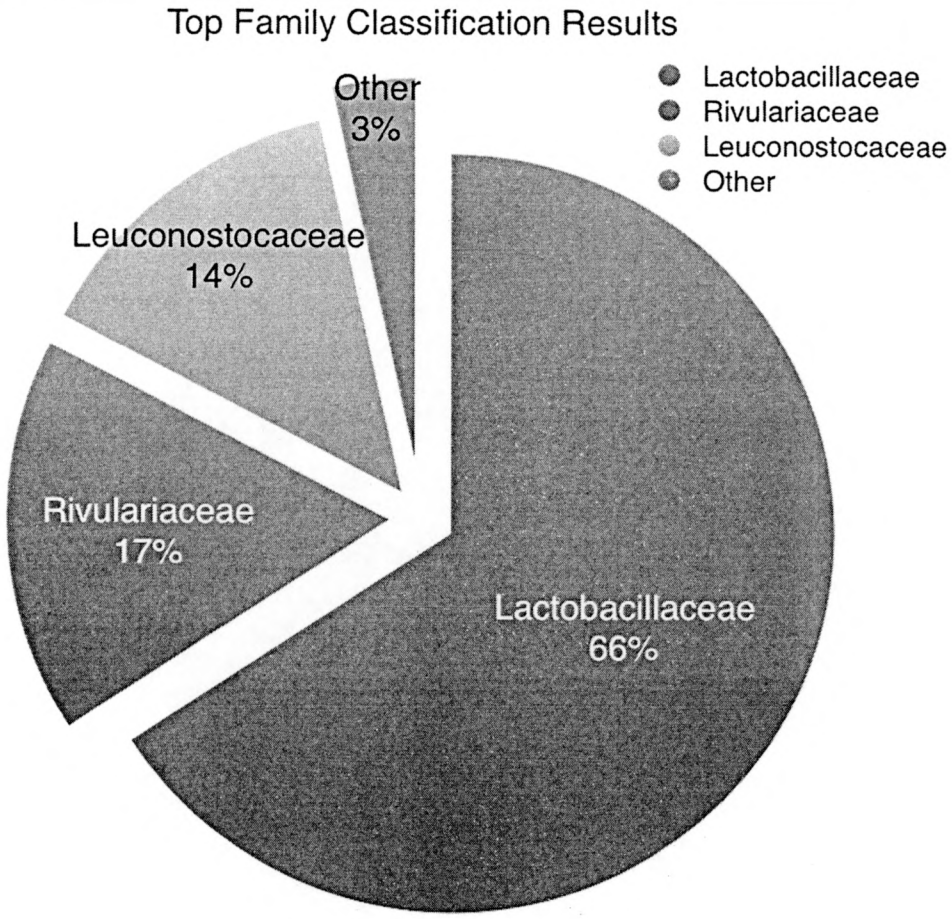
Top Order Classification Results

Classification	% Total Reads	Number of Reads
Lactobacillales	78.96%	1,425,608
Stigonematales	16.92%	301,584
Bacillales	1.32%	24,044
Pseudomonadales	1.28%	23,197
Enterobacteriales	0.75%	13,597
Unclassified at Order level	0.35%	6,291
Vibrionales	0.22%	4,056
Rhodobacterales	0.20%	3,704



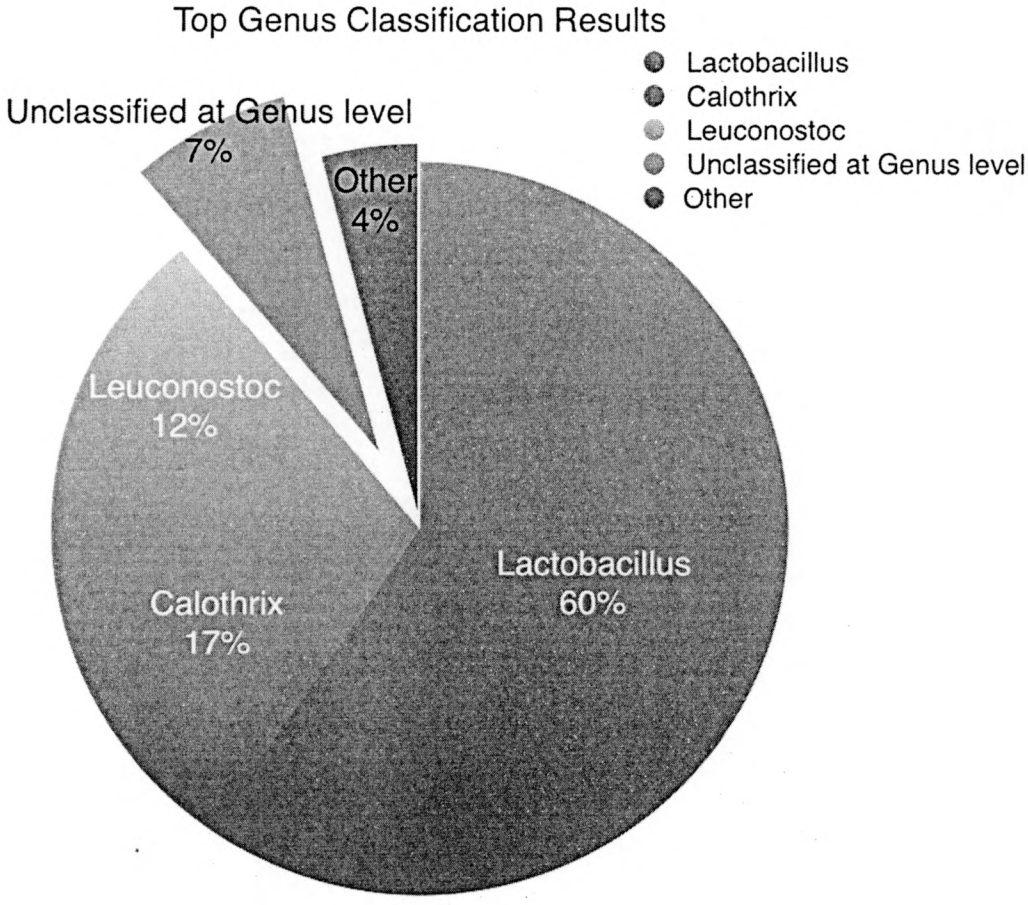
Top Family Classification Results

Classification	% Total Reads	Number of Reads
Lactobacillaceae	65.61%	1,173,824
Rivulariaceae	17.30%	301,584
Leuconostocaceae	13.66%	246,503
Pseudomonadaceae	0.99%	18,016
Bacillaceae	0.94%	17,021
Enterobacteriaceae	0.75%	13,597
Unclassified at Family level	0.44%	8,009
Listeriaceae	0.31%	5,626



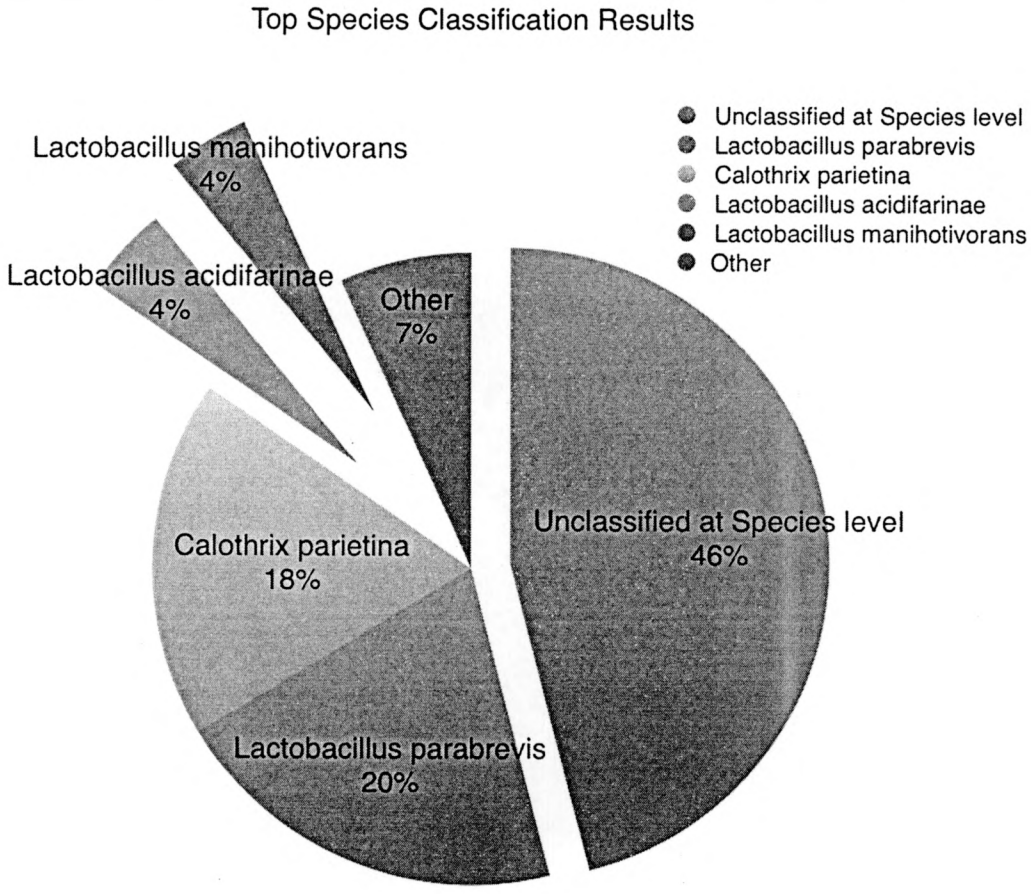
Top Genus Classification Results

Classification	% Total Reads	Number of Reads
Lactobacillus	59.70%	1,048,370
Calothrix	17.20%	301,584
Leuconostoc	12.24%	213,304
Unclassified at Genus level	6.77%	123,001
Weissella	1.82%	32,996
Pseudomonas	0.99%	18,016
Pediococcus	0.73%	13,270
Bacillus	0.55%	9,987



Top Species Classification Results

Classification	% Total Reads	Number of Reads
Unclassified at Species level	46.45%	758,572
Lactobacillus parabrevis	20.47%	335,620
Calothrix parietina	17.90%	301,582
Lactobacillus acidifarinae	4.44%	73,464
Lactobacillus manihotivorans	3.97%	68,473
Lactobacillus japonicus	2.95%	53,682
Weissella viridescens	2.02%	29,361
Lactobacillus plantarum	1.80%	28,153



Experimental 2

Sample Information

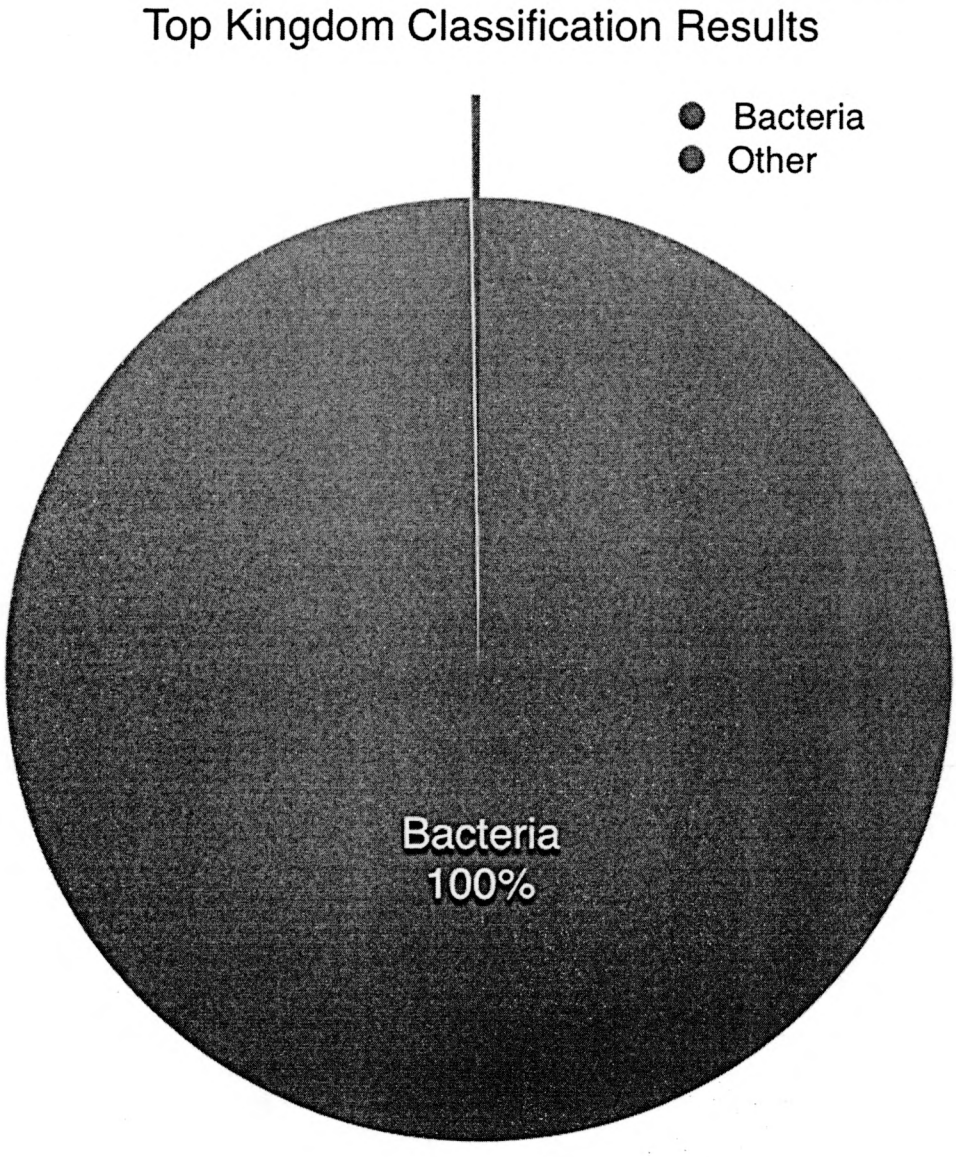
Sample ID:	E2
Sample Name:	JG02-E2
Run Folder:	D:\Illumina\MiSeqAnalysis\7e0729e5f91147ca828ca5ee013f96f5
Taxonomy File:	gg_13_5_species_32bp.dat

Sequencing Statistics

Total Reads	Reads Passing Quality Filtering	% Reads Passing Quality Filtering
2,192,708	2,022,481	92.2 %

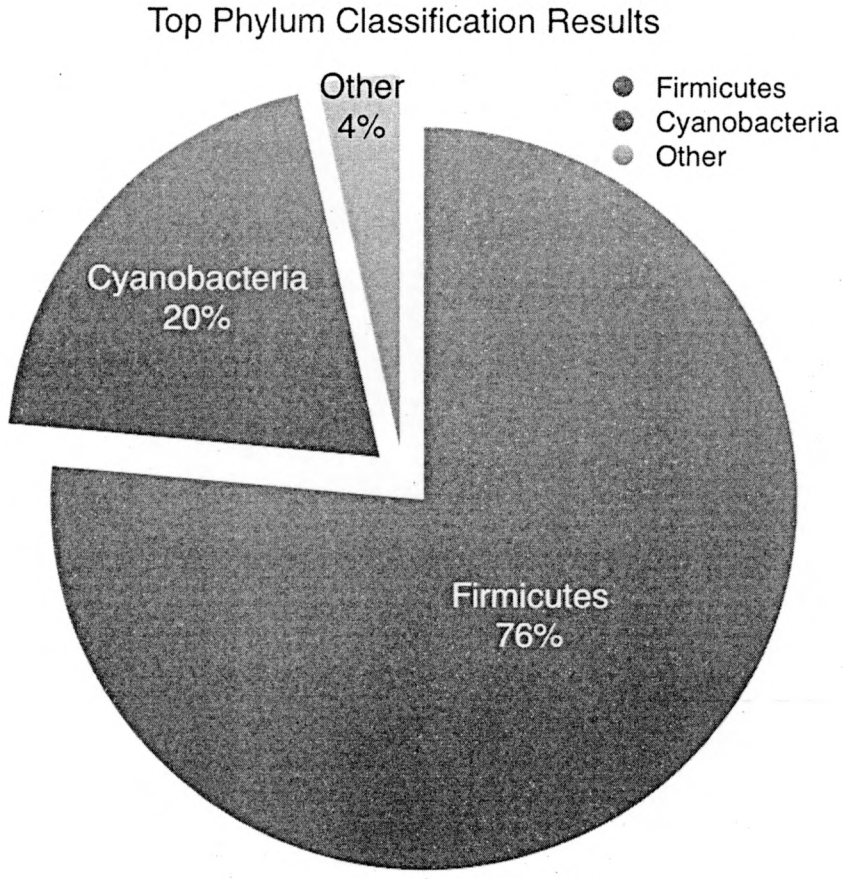
Top Kingdom Classification Results

Classification	% Total Reads	Number of Reads
Bacteria	99.68%	2,015,921
Unclassified at Kingdom level	0.21%	4,275
Archaea	0.11%	2,236
Viruses	0.00%	49



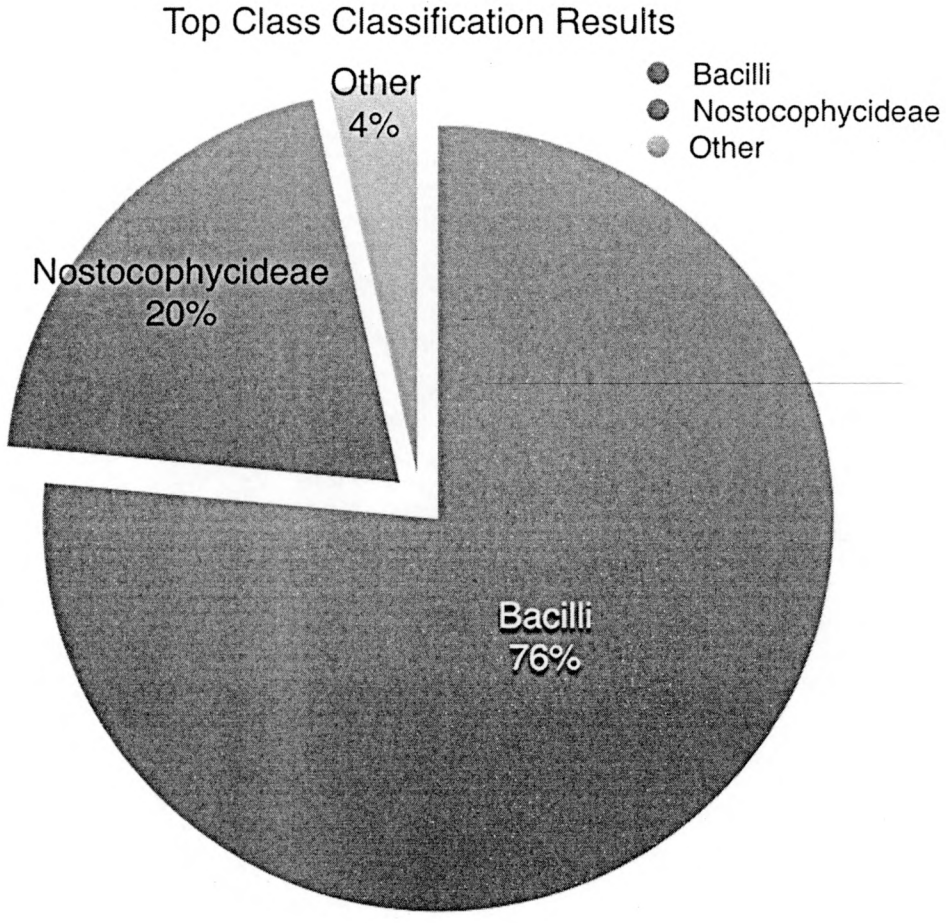
Top Phylum Classification Results

Classification	% Total Reads	Number of Reads
Firmicutes	76.39%	1,544,952
Cyanobacteria	20.01%	404,787
Proteobacteria	2.96%	59,870
Unclassified at Phylum level	0.34%	6,794
Bacteroidetes	0.11%	1,864
Euryarchaeota	0.10%	1,829
Actinobacteria	0.05%	775
Chloroflexi	0.04%	617



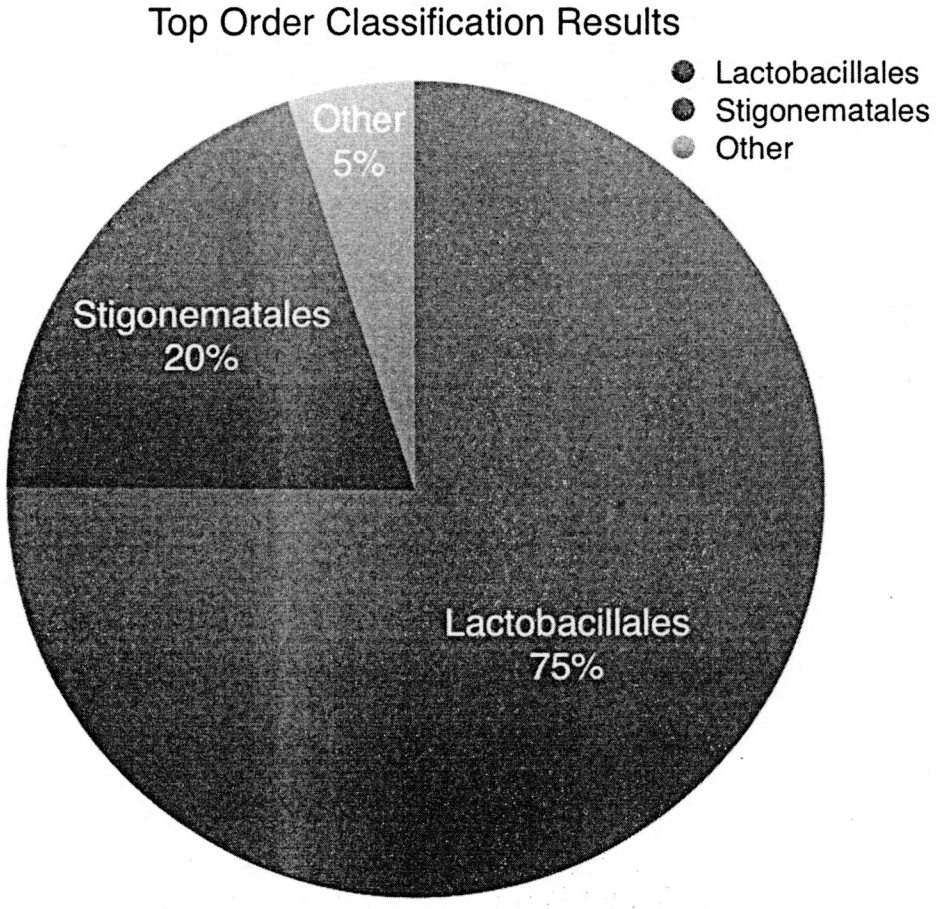
Top Class Classification Results

Classification	% Total Reads	Number of Reads
Bacilli	76.45%	1,542,127
Nostocophycideae	19.93%	403,102
Gammaproteobacteria	2.69%	52,677
Unclassified at Class level	0.38%	7,620
Alphaproteobacteria	0.27%	5,499
Clostridia	0.13%	2,601
Methanomicrobia	0.09%	1,784
Oscillatoriophycideae	0.06%	1,187



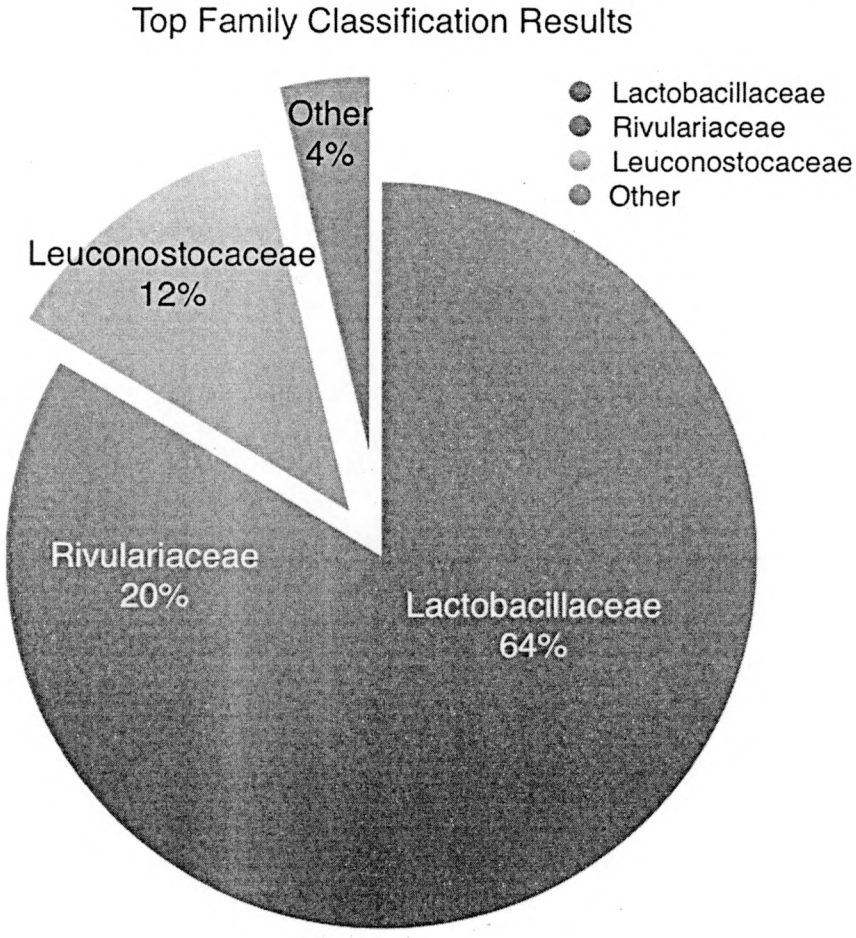
Top Order Classification Results

Classification	% Total Reads	Number of Reads
Lactobacillales	75.34%	1,513,367
Stigonematales	19.72%	398,830
Bacillales	1.41%	28,435
Pseudomonadales	1.76%	27,555
Enterobacteriales	0.87%	17,605
Unclassified at Order level	0.44%	8,925
Vibrionales	0.25%	4,969
Rhodobacterales	0.21%	4,341



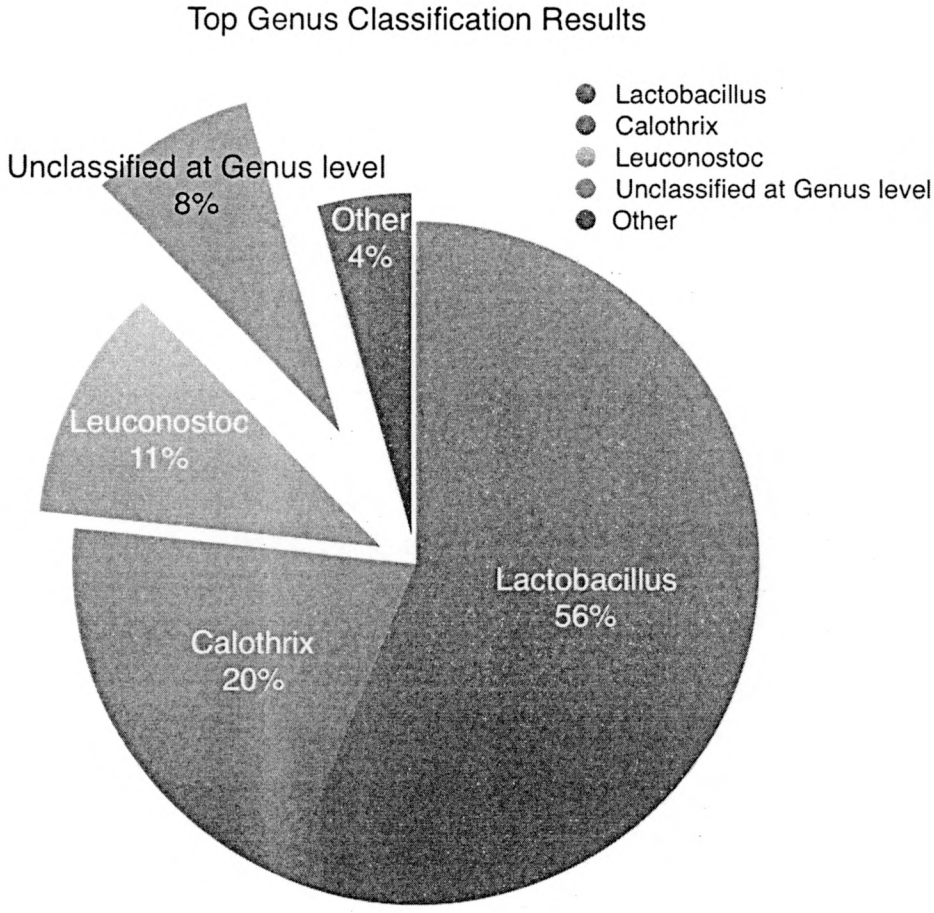
Top Family Classification Results

Classification	% Total Reads	Number of Reads
Lactobacillaceae	64.26%	1,260,188
Rivulariaceae	19.72%	398,830
Leuconostocaceae	12.22%	247,087
Pseudomonadaceae	1.06%	21,363
Bacillaceae	0.96%	19,379
Enterobacteriaceae	0.87%	17,605
Unclassified at Family level	0.54%	10,952
Listeriaceae	0.37%	7,396



Top Genus Classification Results

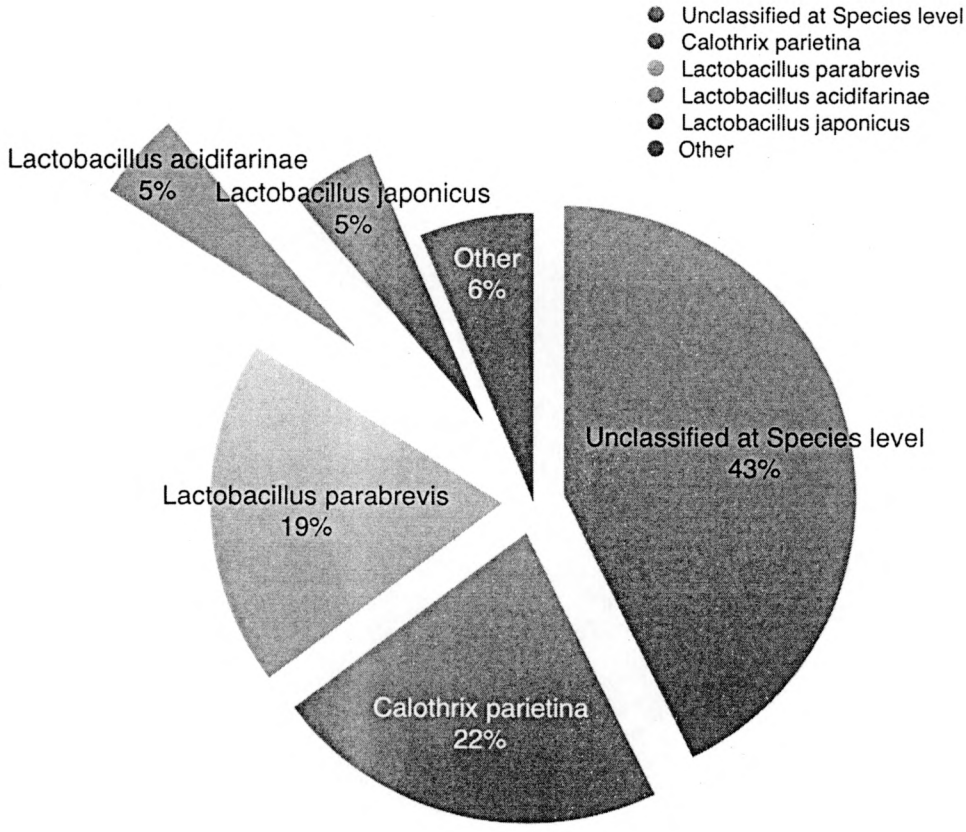
Classification	% Total Reads	Number of Reads
Lactobacillus	56.41%	1,098,353
Calothrix	19.93%	398,830
Leuconostoc	10.94%	217,189
Unclassified at Genus level	8.42%	152,179
Weissella	1.47%	29,685
Pediococcus	1.18%	23,877
Pseudomonas	1.06%	21,362
Candidatus Blochmannia	0.59%	11,883



Top Species Classification Results

Classification	% Total Reads	Number of Reads
Unclassified at Species level	43.36%	775,794
Calothrix parietina	21.72%	398,823
Lactobacillus parabrevis	19.31%	350,085
Lactobacillus acidifarinae	4.98%	88,517
Lactobacillus japonicus	4.85%	85,860
Lactobacillus manihotivorans	3.30%	60,627
Leuconostoc carnosum	1.31%	28,511
Weissella viridescens	1.17%	26,108

Top Species Classification Results



Experimental 3

Sample Information

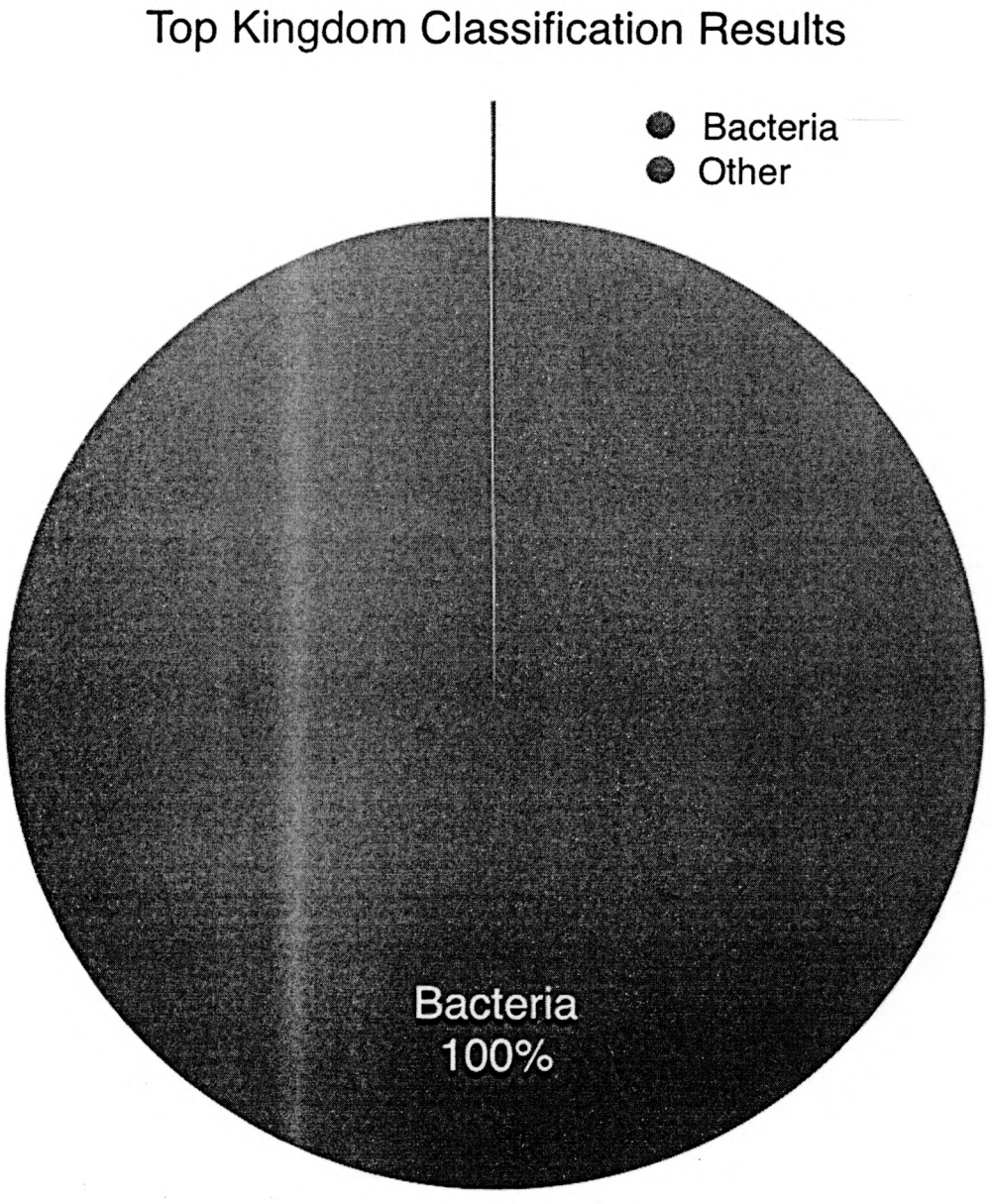
Sample ID:	E3
Sample Name:	JG03-E3
Run Folder:	D:\Illumina\MiSeqAnalysis\7e0729e5f91147ca828ca5ee013f96f5
Taxonomy File:	gg_13_5_species_32bp.dat

Sequencing Statistics

Total Reads	Reads Passing Quality Filtering	% Reads Passing Quality Filtering
2,688,840	2,444,648	90.9 %

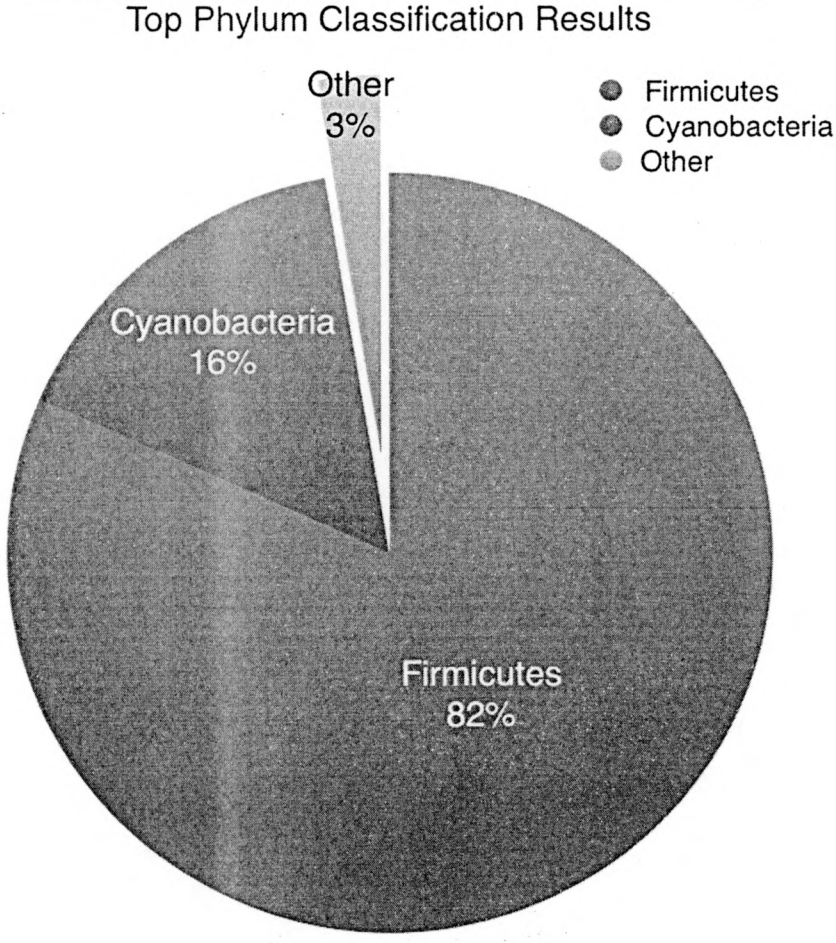
Top Kingdom Classification Results

Classification	% Total Reads	Number of Reads
Bacteria	99.83%	2,440,393
Unclassified at Kingdom level	0.10%	2,354
Archaea	0.07%	1,741
Viruses	0.01%	160



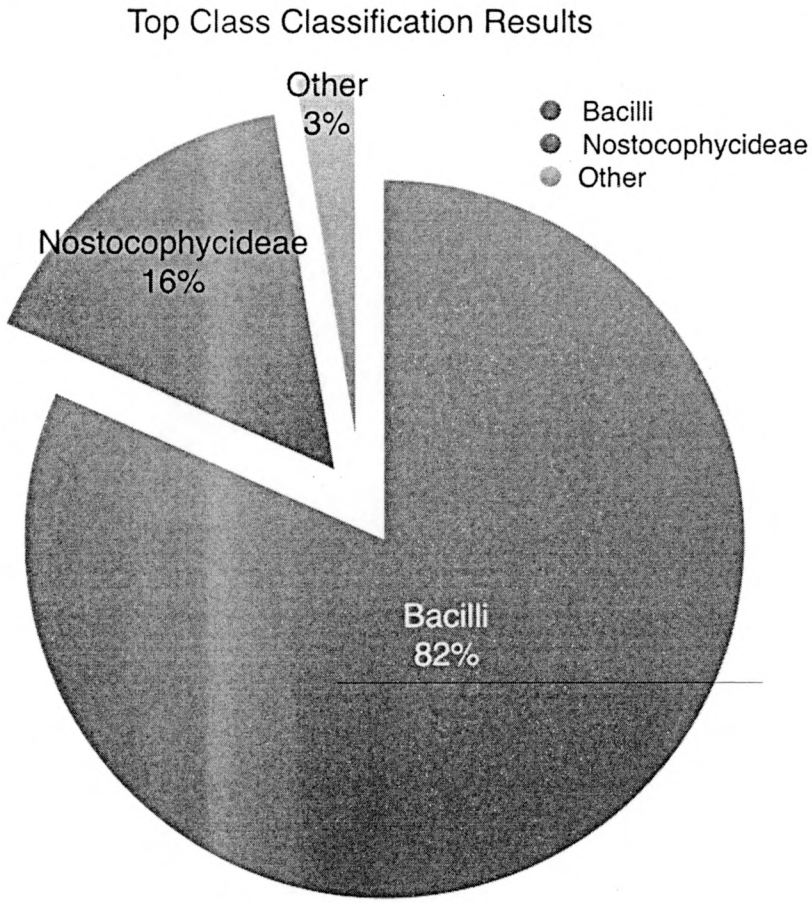
Top Phylum Classification Results

Classification	% Total Reads	Number of Reads
Firmicutes	82.02%	1,995,238
Cyanobacteria	15.31%	382,927
Proteobacteria	2.32%	56,754
Unclassified at Phylum level	0.18%	4,506
Bacteroidetes	0.06%	1,525
Euryarchaeota	0.06%	1,417
Actinobacteria	0.03%	787
Chloroflexi	0.02%	468



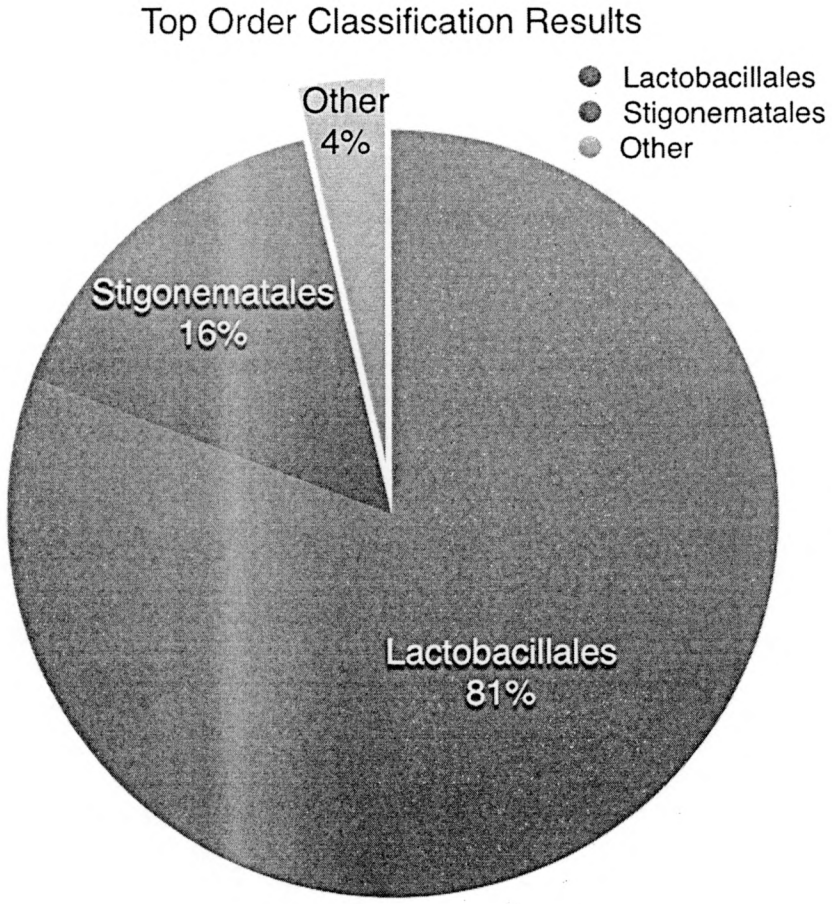
Top Class Classification Results

Classification	% Total Reads	Number of Reads
Bacilli	81.75%	1,993,277
Nostocophycideae	15.60%	381,413
Gammaproteobacteria	2.02%	49,294
Alphaproteobacteria	0.25%	6,199
Unclassified at Class level	0.21%	5,184
Clostridia	0.07%	1,802
Methanomicrobia	0.06%	1,401
Oscillatoriophycideae	0.04%	1,088



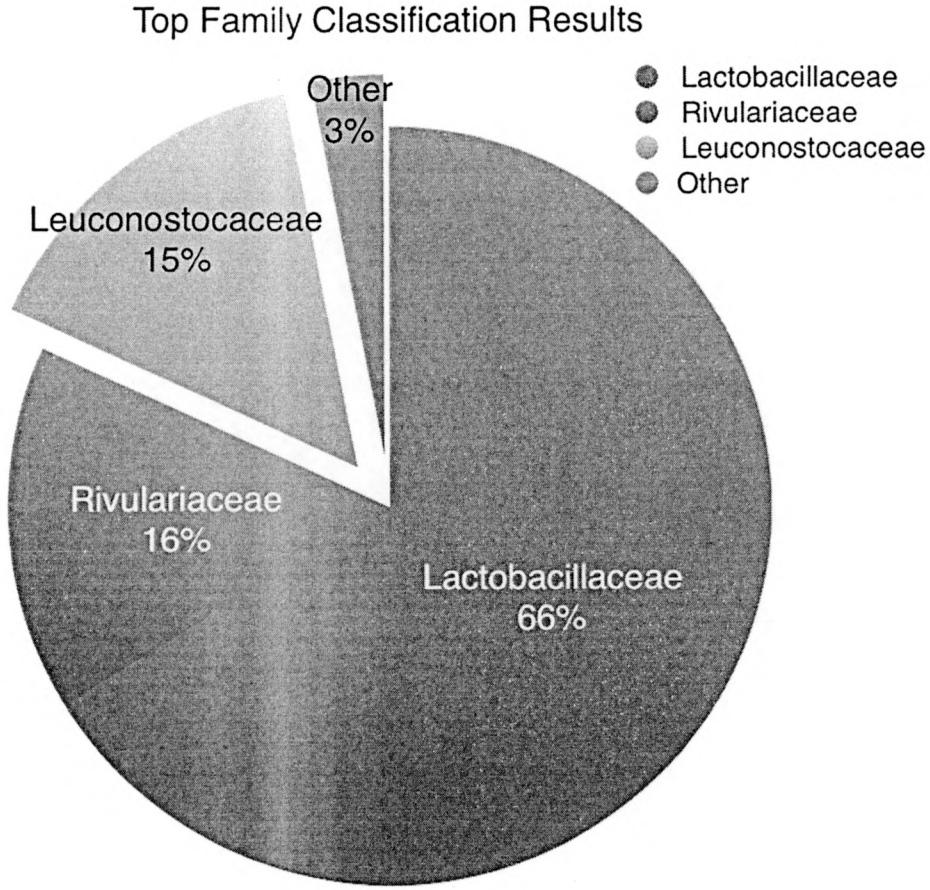
Top Order Classification Results

Classification	% Total Reads	Number of Reads
Lactobacillales	81.05%	1,962,278
Stigonematales	15.51%	376,716
Bacillales	1.15%	30,576
Pseudomonadales	1.09%	29,058
Enterobacteriales	0.54%	13,097
Unclassified at Order level	0.27%	6,521
Vibrionales	0.20%	4,773
Rhodobacterales	0.19%	4,560



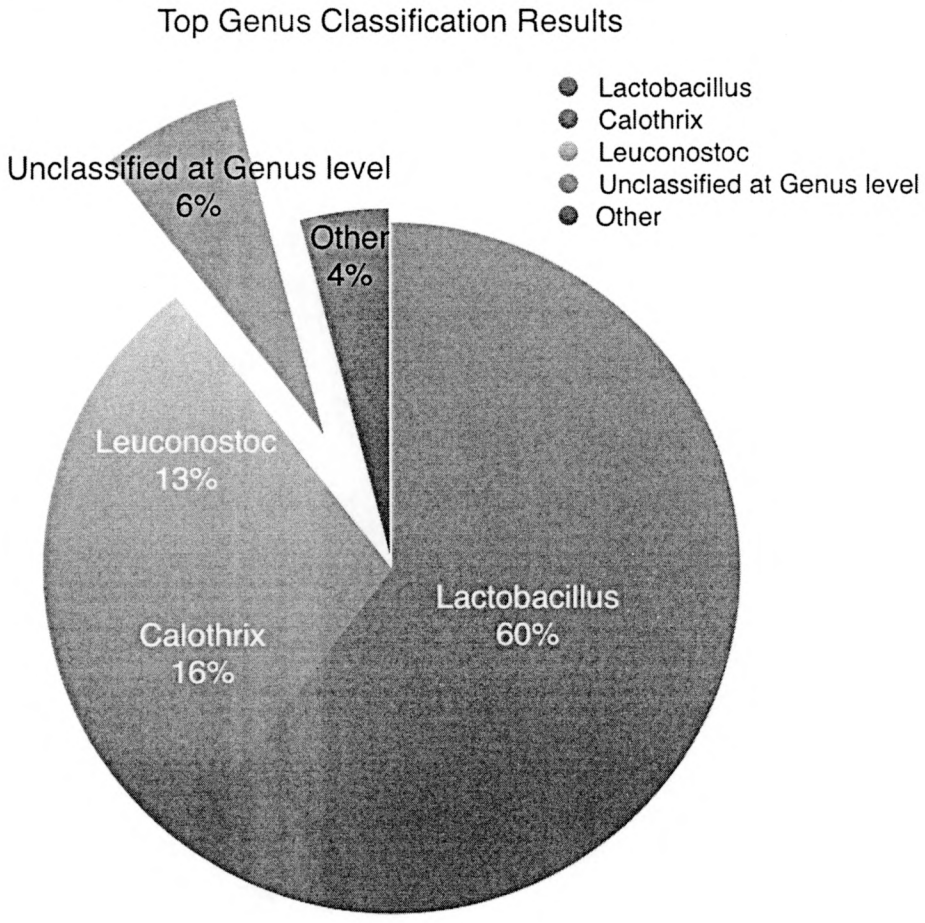
Top Family Classification Results

Classification	% Total Reads	Number of Reads
Lactobacillaceae	66.21%	1,594,065
Rivulariaceae	16.01%	376,716
Leuconostocaceae	14.76%	361,169
Pseudomonadaceae	0.94%	22,958
Bacillaceae	0.84%	20,513
Enterobacteriaceae	0.54%	13,097
Unclassified at Family level	0.36%	8,850
Listeriaceae	0.34%	8,370



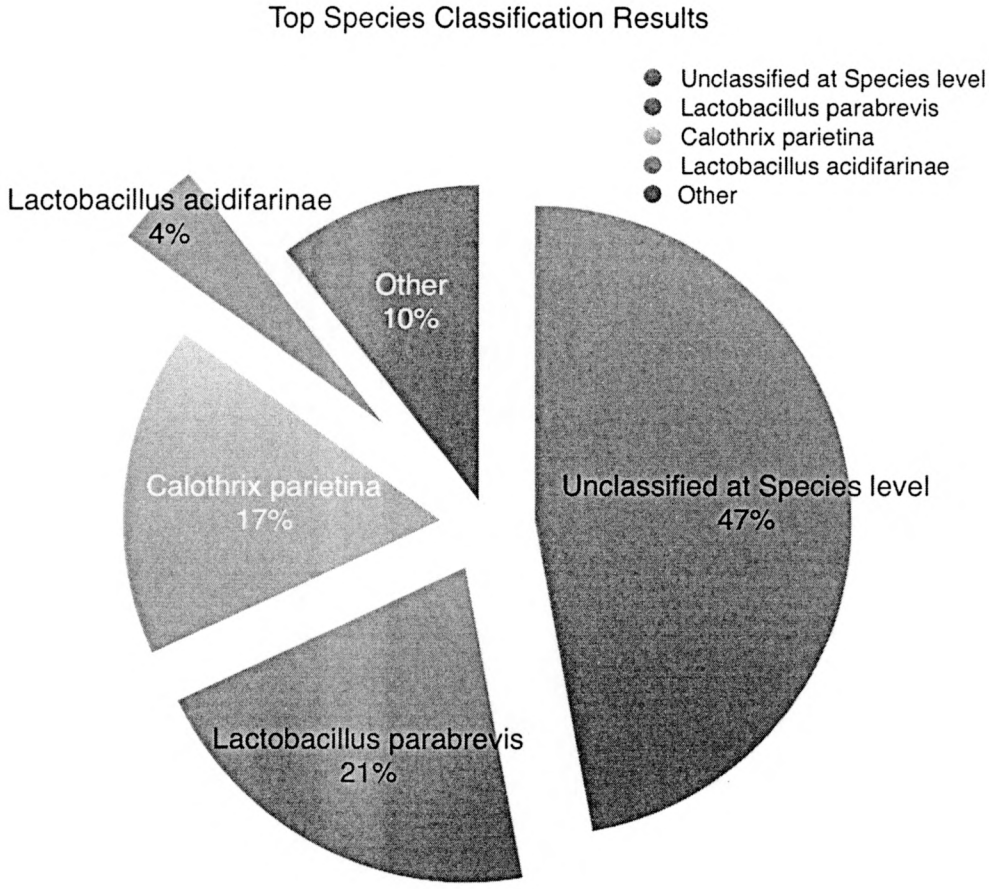
Top Genus Classification Results

Classification	% Total Reads	Number of Reads
Lactobacillus	60.44%	1,434,988
Calothrix	16.32%	376,716
Leuconostoc	12.95%	314,097
Unclassified at Genus level	6.28%	153,568
Weissella	1.91%	46,733
Pseudomonas	0.94%	22,958
Pediococcus	0.70%	17,102
Bacillus	0.46%	11,318



Top Species Classification Results

Classification	% Total Reads	Number of Reads
Unclassified at Species level	47.19%	1,043,577
Lactobacillus parabrevis	20.91%	464,839
Calothrix parietina	17.41%	376,712
Lactobacillus acidifarinae	4.02%	98,169
Lactobacillus manihotivorans	3.49%	76,985
Lactobacillus japonicus	3.48%	76,817
Weissella viridescens	1.85%	41,525
Lactobacillus plantarum	1.65%	37,123



Appendix E: Analysis of NGS Results

We were interested in understanding which bacteria were potentially contributed by the environment of on-street mobile food carts, we generated two lists: the first lists all bacteria that were uniquely found in experimental samples (and **not** in controls); the second lists all those species found uniquely in control groups (and **not** in experimentals). The Experimental list (Table 12) has a total 355 unique bacteria; the Control list (Table 13) has a total of 207 unique bacteria. Both list are in alphabetical order.

Bacteria Present In Experimental NGS (and not in Control NGS)	
1.	*Acetobacter*
2.	*Acetobacter cibinongensis*
3.	*Acetobacter pasteurianus*
4.	*Achromobacter insolitus*
5.	*Acidovorax facilis*
6.	*Acidovorax temperans*
7.	*Acidovorax wohlfahrtii*
8.	*Acinetobacter baylyi*
9.	*Acinetobacter bouvetii*
10.	*Acinetobacter calcoaceticus*
11.	*Acinetobacter gyllenbergii*
12.	*Acinetobacter haemolyticus*
13.	*Acinetobacter oleivorans*
14.	*Acinetobacter parvus*
15.	*Acinetobacter rhizosphaerae*
16.	*Acinetobacter schindleri*

17.	*Acinetobacter ursingii*
18.	*Actinomyces*
19.	*Actinomyces naturae*
20.	*Aerococcus*
21.	*Aerococcus christensenii*
22.	*Aerococcus viridans*
23.	*Agrobacterium tumefaciens*
24.	*Alkalibacillus salilacus*
25.	*Alkalibacterium*
26.	*Anaerobacillus*
27.	*Anaerobacillus alkalilacustre*
28.	*Anoxybacillus*
29.	*Anoxybacillus rupiensis*
30.	*Arthrobacter nicotianae*
31.	*Asticcacaulis*
32.	*Atopobium*
33.	*Atopobium fossor*
34.	*Azospirillum*
35.	*Bacillus alcaliinulinus*
36.	*Bacillus atrophaeus*
37.	*Bacillus badius*
38.	*Bacillus cereus*
39.	*Bacillus coagulans*
40.	*Bacillus djibelorensis*
41.	*Bacillus ginsenggisoli*
42.	*Bacillus hackensackii*

43.	*Bacillus herbersteinensis*
44.	*Bacillus infantis*
45.	*Bacillus lehensis*
46.	*Bacillus malacitensis*
47.	*Bacillus murimartini*
48.	*Bacillus smithii*
49.	*Blautia*
50.	*Brachybacterium arcticum*
51.	*Brachybacterium faecium*
52.	*Brachybacterium squillarum*
53.	*Brevibacterium album*
54.	*Brevundimonas olei*
55.	*Burkholderia*
56.	*Burkholderia vietnamiensis*
57.	*Caloramator*
58.	*Caloramator mitchellensis*
59.	*Candidatus Phlomobacter*
60.	*Candidatus Phlomobacter fragariae*
61.	*Candidatus Portiera*
62.	*Carnobacterium funditum*
63.	*Carnobacterium gallinarum*
64.	*Carnobacterium mobile*
65.	*Cetobacterium somerae*
66.	*Chitinophaga*
67.	*Chitinophaga soli*
68.	*Chryseobacterium bovis*

69.	*Chryseobacterium hispanicum*
70.	*Chryseobacterium isbiliense*
71.	*Chryseobacterium joostei*
72.	*Chryseobacterium molle*
73.	*Chryseobacterium soli*
74.	*Chryseobacterium wanjuense*
75.	*Citrobacter freundii*
76.	*Citrobacter werkmanii*
77.	*Clostridium histolyticum*
78.	*Clostridium longisporum*
79.	*Cohnella soli*
80.	*Cohnella thermotolerans*
81.	*Comamonas composti*
82.	*Corynebacterium falsenii*
83.	*Corynebacterium kroppenstedtii*
84.	*Corynebacterium testudinoris*
85.	*Corynebacterium variabile*
86.	*Curtobacterium flaccumfaciens*
87.	*Deinococcus aeria*
88.	*Deinococcus alpinitundrae*
89.	*Deinococcus altitudinis*
90.	*Deinococcus gobiensis*
91.	*Deinococcus marmoris*
92.	*Deinococcus radiodurans*
93.	*Dermacoccus*
94.	*Desulfosporosinus*

95.	*Dethiosulfovibrio*
96.	*Dickeya dadantii*
97.	*Emticicia*
98.	*Emticicia oligotrophica*
99.	*Enhydrobacter aerosaccus*
100.	*Enterobacter aceae*
101.	*Enterobacter aerogenes*
102.	*Enterobacter cloacae*
103.	*Enterobacter gergoviae*
104.	*Enterobacter ludwigii*
105.	*Enterobacter nickellidurans*
106.	*Enterococcus azikeevi*
107.	*Enterococcus faecalis*
108.	*Enterococcus gilvus*
109.	*Enterococcus hawaiiensis*
110.	*Enterococcus silesiacus*
111.	*Erwinia billingiae*
112.	*Erwinia dispersa*
113.	*Erwinia papayae*
114.	*Erwinia rhapontici*
115.	*Erythrobacter*
116.	*Erythrobacter aquimaris*
117.	*Exiguobacterium*
118.	*Exiguobacterium acetylicum*
119.	*Exiguobacterium indicum*
120.	*Flavobacterium gelidilacus*

121.	*Flectobacillus*
122.	*Fructobacillus*
123.	*Fructobacillus ficulneus*
124.	*Fructobacillus fructosus*
125.	*Fructobacillus pseudoficulneus*
126.	*Fusobacterium gonidiaformans*
127.	*Fusobacterium naviforme*
128.	*Geobacillus*
129.	*Geobacillus bogazici*
130.	*Geobacillus caldoxylosilyticus*
131.	*Geobacillus gargensis*
132.	*Geobacillus jurassicus*
133.	*Geobacillus kaustophilus*
134.	*Geobacillus stearothermophilus*
135.	*Geobacillus thermoglucosidans*
136.	*Geobacillus thermoparaffinivorans*
137.	*Geobacillus toebii*
138.	*Geobacillus vulcani*
139.	*Geobacillus zalihae*
140.	*Geobacter*
141.	*Geobacter toluenoxydans*
142.	*Gluconobacter japonicus*
143.	*Halanaerobacter*
144.	*Hydrogenophilus*
145.	*Hydrogenophilus hirschii*
146.	*Janibacter*

147.	*Jeotgalicoccus*
148.	*Jeotgalicoccus coquinae*
149.	*Klebsiella granulomatis*
150.	*Klebsiella pneumoniae*
151.	*Klebsiella variicola*
152.	*Kocuria*
153.	*Kocuria gwangalliensis*
154.	*Kocuria kristinae*
155.	*Kocuria palustris*
156.	*Kocuria rhizophila*
157.	*Kocuria rosea*
158.	*Kurthia*
159.	*Kurthia gibsonii*
160.	*Lactobacillus acetotolerans*
161.	*Lactobacillus antri*
162.	*Lactobacillus brevis*
163.	*Lactobacillus casei*
164.	*Lactobacillus delbrueckii*
165.	*Lactobacillus equicursoris*
166.	*Lactobacillus fabifermentans*
167.	*Lactobacillus farciminis*
168.	*Lactobacillus fermentum*
169.	*Lactobacillus fructivorans*
170.	*Lactobacillus guizhouensis*
171.	*Lactobacillus hammesii*
172.	*Lactobacillus hayakitensis*

173.	*Lactobacillus helveticus*
174.	*Lactobacillus homohiochii*
175.	*Lactobacillus intestinalis*
176.	*Lactobacillus letivazi*
177.	*Lactobacillus namurensis*
178.	*Lactobacillus nodensis*
179.	*Lactobacillus oligofermentans*
180.	*Lactobacillus paracasei*
181.	*Lactobacillus parafarraginis*
182.	*Lactobacillus pentosus*
183.	*Lactobacillus pontis*
184.	*Lactobacillus rhamnosus*
185.	*Lactobacillus rossiae*
186.	*Lactobacillus secaliphilus*
187.	*Lactobacillus similis*
188.	*Lactobacillus taiwanensis*
189.	*Lactobacillus thailandensis*
190.	*Lactobacillus tucceti*
191.	*Lactobacillus zeae*
192.	*Lactobacillus zymae*
193.	*Lactococcus fujiensis*
194.	*Lactococcus garvieae*
195.	*Lentibacillus kapialis*
196.	*Lentzea*
197.	*Leucobacter*
198.	*Leuconostoc argentinum*

199.	*Leuconostoc citreum*
200.	*Leuconostoc garlicum*
201.	*Leuconostoc inhae*
202.	*Leuconostoc mesenteroides*
203.	*Leuconostoc palmae*
204.	*Leuconostoc pseudomesenteroides*
205.	*Limnohabitans*
206.	*Listeria*
207.	*Listeria innocua*
208.	*Lysinibacillus*
209.	*Macrococcus brunensis*
210.	*Macrococcus caseolyticus*
211.	*Macrococcus equipercicus*
212.	*Marinitoga*
213.	*Marinomonas arenicola*
214.	*Marinomonas primoryensis*
215.	*Marinomonas rhizomae*
216.	*Meiothermus*
217.	*Melissococcus*
218.	*Methylobacterium dankookense*
219.	*Methylobacterium goesingense*
220.	*Methylobacterium mesophilicum*
221.	*Methylobacterium radiotolerans*
222.	*Microbacterium*
223.	*Microbacterium chocolatum*
224.	*Moraxella*

225.	*Morganella*
226.	*Morganella psychrotolerans*
227.	*Mycetocola*
228.	*Myroides injenensis*
229.	*Myroides odoratimimus*
230.	*Myroides odoratus*
231.	*Negativicoccus*
232.	*Negativicoccus succinicivorans*
233.	*Nesterenkonia terrae*
234.	*Ochrobactrum pseudogrignonense*
235.	*Oenococcus*
236.	*Olivibacter*
237.	*Oscillospira eae*
238.	*Paenibacillus alginolyticus*
239.	*Paenisporosarcina*
240.	*Paenisporosarcina quisquiliarum*
241.	*Paracoccus homiensis*
242.	*Paracoccus marcusii*
243.	*Pediococcus acidilactici*
244.	*Pediococcus inopinatus*
245.	*Pediococcus stilesii*
246.	*Pelotomaculum*
247.	*Pelotomaculum isophthalicicum*
248.	*Peptoniphilus asaccharolyticus*
249.	*Pigmentiphaga*
250.	*Pigmentiphaga kullae*

251.	*Planococcus*
252.	*Planococcus maritimus*
253.	*Planomicrobium*
254.	*Prevotella paludivivens*
255.	*Propionicimonas*
256.	*Propionigenium*
257.	*Propionigenium modestum*
258.	*Propionispora*
259.	*Propionispora hippei*
260.	*Proteus penneri*
261.	*Providencia burhodogranariea*
262.	*Providencia rettgeri*
263.	*Pseudoalteromonas gracilis*
264.	*Pseudoalteromonas haloplanktis*
265.	*Pseudoalteromonas issachenkonii*
266.	*Pseudomonas aeruginosa*
267.	*Pseudomonas cichorii*
268.	*Pseudomonas cinnamophila*
269.	*Pseudomonas cremoricolorata*
270.	*Pseudomonas mendocina*
271.	*Pseudomonas monteilii*
272.	*Pseudomonas plecoglossicida*
273.	*Pseudomonas tropicalis*
274.	*Psychrobacter alimentarius*
275.	*Psychrobacter cibarius*
276.	*Psychrobacter fozii*

277.	*Psychrobacter immobilis*
278.	*Psychrobacter namhaensis*
279.	*Psychrobacter phenylpyruvicus*
280.	*Psychrobacter vallis*
281.	*Pullulanibacillus*
282.	*Pullulanibacillus naganoensis*
283.	*Rathayibacter*
284.	*Rathayibacter tritici*
285.	*Rhodobacter ovatus*
286.	*Rhodoferax*
287.	*Rhodoferax ferrireducens*
288.	*Rickettsia monacensis*
289.	*Roseomonas*
290.	*Rothia*
291.	*Ruegeria*
292.	*Ruegeria lacuscaerulensis*
293.	*Saccharopolyspora*
294.	*Salinibacterium*
295.	*Salinicoccus*
296.	*Salinicoccus iranensis*
297.	*Salinicoccus luteus*
298.	*Sejongia*
299.	*Shewanella frigidimarina*
300.	*Shewanella halifaxensis*
301.	*Shewanella livingstonensis*
302.	*Shewanella morhuae*

303.	*Shewanella profunda*
304.	*Shewanella vesiculosa*
305.	*Shinella*
306.	*Sphingobacterium multivorum*
307.	*Sphingomonas echinoides*
308.	*Sphingomonas insulae*
309.	*Sphingomonas melonis*
310.	*Sporolactobacillus*
311.	*Sporolactobacillus putidus*
312.	*Sporotomaculum*
313.	*Sporotomaculum syntrophicum*
314.	*Staphylococcus arlettae*
315.	*Staphylococcus equorum*
316.	*Staphylococcus sciuri*
317.	*Staphylococcus succinus*
318.	*Staphylococcus vitulinus*
319.	*Staphylococcus xylosus*
320.	*Stenotrophomonas acidaminiphila*
321.	*Stenotrophomonas geniculata*
322.	*Stenotrophomonas koreensis*
323.	*Stenotrophomonas maltophilia*
324.	*Stenotrophomonas pavanii*
325.	*Streptococcus thermophilus*
326.	*Telmatospirillum*
327.	*Telmatospirillum siberiense*
328.	*Tetragenococcus*

329.	*Tetragenococcus doogicus*
330.	*Thalassospira*
331.	*Thermicanus*
332.	*Thermomonas*
333.	*Thermomonas fusca*
334.	*Trabulsiella farmeri*
335.	*Trabulsiella odontotermitis*
336.	*Trichococcus*
337.	*Vagococcus carniphilus*
338.	*Vagococcus penaei*
339.	*Vagococcus salmoninarum*
340.	*Variovorax*
341.	*Variovorax paradoxus*
342.	*Vibrio casei*
343.	*Vibrio comitans*
344.	*Vogesella*
345.	*Vogesella perlucida*
346.	*Wautersiella*
347.	*Wautersiella falsenii*
348.	*Weissella confusa*
349.	*Weissella hanii*
350.	*Weissella paramesenteroides*
351.	*Weissella thailandensis*
352.	*Weissella viridescens*
353.	*Zoogloea*
354.	*Zoogloea oryzae*

355.	*Zoogloea resiniphila*

Table 14: This table contains an alphabetical listing of all bacteria that are found in the experimental master list, but are not represented in the control master list.

Bacteria Present In Control NGS (not in Experimental NGS)	
1.	*Acidianus*
2.	*Acidovorax caeni*
3.	*Acinetobacter xiamenensis*
4.	*Actinotalea*
5.	*Actinotalea fermentans*
6.	*Alishewanella*
7.	*Alishewanella agri*
8.	*Aminiphilus*
9.	*Aminiphilus circumscriptus*
10.	*Anaerolinea*
11.	*Anaerolinea thermolimosa*
12.	*Aquabacterium*
13.	*Arthrobacter psychrophenolicus*
14.	*Arthrobacter stackebrandtii*
15.	*Arthrobacter sulfureus*
16.	*Azoarcus*
17.	*Bacillus clausii*
18.	*Bacillus flexus*
19.	*Bacillus safensis*
20.	*Bacillus shandongensis*
21.	*Bacteroides heparinolyticus*

22.	*Bdellovibrio*
23.	*Bdellovibrio exovorus*
24.	*Bifidobacterium subtile*
25.	*Caldithrix*
26.	*Caldithrix palaeochoryensis*
27.	*Candidatus Methylacidiphilum*
28.	*Carboxydocella*
29.	*Carboxydocella ferrireducens*
30.	*Cellulomonas*
31.	*Chondromyces*
32.	*Chondromyces pediculatus*
33.	*Chroococcus*
34.	*Chroococcus minutus*
35.	*Clostridium haemolyticum*
36.	*Clostridium perfringens*
37.	*Corynebacterium amycolatum*
38.	*Corynebacterium genitalium*
39.	*Corynebacterium glucuronolyticum*
40.	*Corynebacterium minutissimum*
41.	*Corynebacterium pseudogenitalium*
42.	*Cupriavidus*
43.	*Cupriavidus pauculus*
44.	*Curtobacterium pusillum*
45.	*Cyanobacterium*
46.	*Cyanobacterium aponinum*
47.	*Deferribacter*

48.	*Deferribacter autotrophicus*
49.	*Delftia*
50.	*Delftia lacustris*
51.	*Desulfobacter*
52.	*Desulfofrigus*
53.	*Desulfofrigus oceanense*
54.	*Desulfosarcina*
55.	*Desulfovibrio psychrotolerans*
56.	*Desulfuromonas*
57.	*Desulfuromonas svalbardensis*
58.	*Desulfuromusa*
59.	*Desulfuromusa succinoxidans*
60.	*Dietzia*
61.	*Dietzia alimentaria*
62.	*Dysgonomonas*
63.	*Dysgonomonas wimpennyi*
64.	*Enterococcus casseliflavus*
65.	*Enterococcus gallinarum*
66.	*Facklamia*
67.	*Fervidobacterium*
68.	*Filifactor*
69.	*Filifactor alocis*
70.	*Flavobacterium antarcticum*
71.	*Flavobacterium glycines*
72.	*Flavobacterium granuli*
73.	*Flavobacterium reichenbachii*

74.	*Francisella*
75.	*Francisella hispaniensis*
76.	*Fusobacterium necrophorum*
77.	*Gallionella*
78.	*Gallionella ferruginea*
79.	*Gemella*
80.	*Gemella haemolysans*
81.	*Gemella sanguinis*
82.	*Gluconobacter krungthepensis*
83.	*Granulicatella*
84.	*Haloanella*
85.	*Haloanella gallinarum*
86.	*Halomonas alkaliantarctica*
87.	*Halomonas campaniensis*
88.	*Halomonas fontilapidosi*
89.	*Halomonas hydrothermalis*
90.	*Helcococcus*
91.	*Helcococcus ovis*
92.	*Herbaspirillum magnetovibrio*
93.	*Hydrogenophaga*
94.	*Hymenobacter xinjiangensis*
95.	*Hyphomicrobium*
96.	*Hyphomicrobium zavarzinii*
97.	*Hyphomonas taiwanensis*
98.	*Isoptericola*
99.	*Kosmotoga*

100.	*Kosmotoga arenicorallina*
101.	*Kushneria indalinina*
102.	*Leucothrix*
103.	*Leucothrix mucor*
104.	*Lewinella marina*
105.	*Litoricola*
106.	*Luteibacter*
107.	*Luteibacter anthropi*
108.	*Luteococcus*
109.	*Luteococcus japonicus*
110.	*Marinobacter santoriniensis*
111.	*Marinomonas protea*
112.	*Marinomonas ushuaiensis*
113.	*Megasphaera*
114.	*Megasphaera hominis*
115.	*Methanosaeta*
116.	*Methanosaeta pelagica*
117.	*Methylonatrum*
118.	*Methylonatrum kenyense*
119.	*Methylophaga thiooxydans*
120.	*Methylotenera*
121.	*Methylotenera mobilis*
122.	*Methyloversatilis*
123.	*Microbulbifer*
124.	*Microcystis*
125.	*Microcystis panniformis*

126.	*Mycobacterium*
127.	*Mycoplasma haemobos*
128.	*Mycoplasma haemominutum*
129.	*Nannocystis aggregans*
130.	*Nevskia*
131.	*Nevskia ramosa*
132.	*Nitrospira*
133.	*Nocardioides islandensis*
134.	*Novosphingobium hassiacum*
135.	*Ochrobactrum thiophenivorans*
136.	*Parascardovia*
137.	*Pedobacter himalayensis*
138.	*Pelomonas*
139.	*Peptococcus*
140.	*Peptococcus niger*
141.	*Photobacterium frigidiphilum*
142.	*Photobacterium ganghwense*
143.	*Photobacterium leiognathi*
144.	*Phyllobacterium*
145.	*Phyllobacterium bourgognense*
146.	*Planctomyces*
147.	*Planifilum*
148.	*Planifilum fimeticola*
149.	*Polynucleobacter*
150.	*Porphyromonas canis*
151.	*Porphyromonas catoniae*

152.	*Propionibacterium avidum*
153.	*Propionibacterium granulosum*
154.	*Propionibacterium microaerophilum*
155.	*Pseudomonas abietaniphila*
156.	*Pseudomonas amygdali*
157.	*Pseudomonas borealis*
158.	*Pseudomonas brassicacearum*
159.	*Pseudomonas cannabina*
160.	*Pseudomonas chloritidismutans*
161.	*Pseudomonas corrugata*
162.	*Pseudomonas frederiksbergensis*
163.	*Pseudomonas gessardii*
164.	*Pseudomonas lini*
165.	*Pseudomonas meliae*
166.	*Pseudomonas orientalis*
167.	*Pseudomonas panacis*
168.	*Pseudomonas thermotolerans*
169.	*Psychrobacter submarinus*
170.	*Rhodobacter blasticus*
171.	*Rhodobacter gluconicum*
172.	*Rhodococcus phenolicus*
173.	*Rhodocyclus*
174.	*Rhodocyclus purpureus*
175.	*Rhodoplanes*
176.	*Rhodovulum*
177.	*Rhodovulum iodosum*

178.	*Rickettsia helvetica*
179.	*Roseivivax*
180.	*Roseivivax halodurans*
181.	*Rubellimicrobium*
182.	*Rubritalea*
183.	*Rubrivivax gelatinosus*
184.	*Ruminococcus*
185.	*Schlegelella*
186.	*Schlegelella aquatica*
187.	*Sediminibacterium*
188.	*Selenomonas*
189.	*Selenomonas infelix*
190.	*Shewanella algae*
191.	*Shewanella olleyana*
192.	*Sphingomonas asaccharolytica*
193.	*Staphylococcus pettenkoferi*
194.	*Staphylococcus pseudolugdunensis*
195.	*Streptococcus equinus*
196.	*Streptococcus oralis*
197.	*Streptococcus tigurinus*
198.	*Streptomyces lazureus*
199.	*Tepidimonas*
200.	*Thermogemmatispora foliorum*
201.	*Thermus*
202.	*Thiomonas*
203.	*Thiomonas thermosulfata*

204.	*Tsukamurella*
205.	*Tsukamurella carboxydivorans*
206.	*Vibrio porteresiae*
207.	*Xanthomonas oryzae*

Table 15: This table contains an alphabetical listing of all bacteria that are found in the control master list, but are not represented in the experimental master list.

In an effort to sort through the dataset and determine which bacteria present in these samples pose a risk to human safety. We have compiled a list of 200 bacteria that are known human pathogens. Cross-listing our master lists of both control and experimental bacteria, we have produce the following lists. Table 14 lists the bacteria in our NGS Experimental master list that also are known human pathogens (N=32; Table 14). The following table (Table 15) lists the bacteria in our NGS Control master list that also are known human pathogens (N=22). In both cases the number of reads produced in the NGS sequencing is also included.

List of Pathogenic Bacteria in Experimental Master List	**Number of Reads**
Pseudomonas	62336
Acinetobacter	12742
Corynebacterium	632
Streptococcus	521
Klebsiella granulomatis	251
Acinetobacter baumannii	249
Sphingomonas	145

Klebsiella pneumoniae	138
Morganella	135
Leuconostoc pseudomesenteroides	130
Yersinia pestis	112
Serratia marcescens	87
Bacillus subtilis	75
Citrobacter	57
Propionibacterium acnes	55
Escherichia coli	37
Klebsiella oxytoca	26
Staphylococcus aureus	21
Prevotella	20
Acinetobacter lwoffii	19
Stenotrophomonas maltophilia	18
Achromobacter	12
Enterobacter aerogenes	12
Enterobacter cloacae	11
Providencia rettgeri	11
Yersinia enterocolitica	11
Brevundimonas diminuta	9
Bacillus cereus	7
Bacteroides	7
Pseudomonas aeruginosa	7
Streptomyces	6
Enterococcus faecalis	3

Table 16: This table includes all the bacteria from the experimental master list that are considered to be pathogenic to humans. The number of times the bacteria was found during the NGS analysis is also found on this table.

List of Pathogenic bacteria in Control Master List	Number of Reads
Pseudomonas	582026
Acinetobacter	14889
Streptococcus	878
Corynebacterium	748
Propionibacterium acnes	738
Sphingomonas	203
Yersinia pestis	127
Prevotella	104
Clostridium perfringens	73
Escherichia coli	50
Staphylococcus aureus	39
Bacteroides	38
Klebsiella oxytoca	32
Acinetobacter lwoffii	26
Acinetobacter baumannii	23
Serratia marcescens	20
Streptomyces	17
Bacillus subtilis	13
Achromobacter	10
Citrobacter	8
Brevundimonas diminuta	6
Yersinia enterocolitica	4

Table 17: This table includes all the bacteria from the control master list that are considered to be pathogenic to humans. The number of 16S sequence reads found for each organism using NGS is also listed.

It is interesting to note that the same four top hits of pathogenic bacteria (*Acinetobacter, Corynebacterium, Pseudomonas, and Streptococcus)* are the same for both the Experimental and Control samples.

In an effort to understand the potential source of bacteria found in both experimental and control samples, we compared the master lists of bacteria generated by NGS to the list of bacteria found in the NIH Human Microbiome Project (NIHHMP) website (http://hmpdacc.org). This list catalogs the common microbial populations present in, and associated with, various parts of the human body.

Table 15 lists, in alphabetical order, the bacteria identified on the Experimental master list that were identified as part of the human microbiome (NIH Human Microbiome Project (NIHHMP)). Also listed on this table is the associated body site for the bacterium. This data is also graphically represented in Figure X following this table. Of the 79 Experimental species that matched the HMP dataset, the proportions were greatest for the GI-tract (54%), oral (16%), skin (13%) and urogenital tract (4%). The entire circle represents 100% of the 79 species, and the sizes of each color represent the proportion of each type of bacteria.

Bacteria from Experimental Master List	**Human Body Sites**
Acinetobacter	Skin
Acinetobacter baumannii	Skin
Acinetobacter calcoaceticus	Skin

Acinetobacter haemolyticus	Airways
Acinetobacter johnsonii	Skin
Acinetobacter junii	Gastrointestinal_tract
Acinetobacter lwoffii	Skin
Acinetobacter radioresistens	Gastrointestinal_tract
Actinomyces	Oral
Aerococcus viridans	Urogenital_tract
Atopobium	Urogenital_tract
Bacillus	Gastrointestinal_tract
Bacillus smithii	Gastrointestinal_tract
Bacteroides	Gastrointestinal_tract
Bifidobacterium	Gastrointestinal_tract
Brevundimonas diminuta	Oral
Citrobacter	Gastrointestinal_tract
Citrobacter freundii	Gastrointestinal_tract
Clostridium	Gastrointestinal_tract
Corynebacterium	Gastrointestinal_tract
Corynebacterium ammoniagenes	Gastrointestinal_tract
Corynebacterium tuberculostearicum	Skin
Dermacoccus	Skin
Desulfovibrio	Gastrointestinal_tract
Enhydrobacter aerosaccus	Skin
Enterobacter cancerogenus	Gastrointestinal_tract
Enterobacter cloacae	Gastrointestinal_tract
Enterobacter hormaechei	Oral
Enterococcus	Gastrointestinal_tract

Enterococcus faecalis	Blood
Escherichia	Gastrointestinal_tract
Escherichia coli	Gastrointestinal_tract
Fusobacterium	Oral
Fusobacterium gonidiaformans	Gastrointestinal_tract
Haemophilus	Oral
Haemophilus parainfluenzae	Skin
Klebsiella	Gastrointestinal_tract
Klebsiella oxytoca	Unknown
Klebsiella pneumoniae	Gastrointestinal_tract
Lactobacillus	Gastrointestinal_tract
Lactobacillus antri	Gastrointestinal_tract
Lactobacillus brevis	Gastrointestinal_tract
Lactobacillus delbrueckii	Gastrointestinal_tract
Lactobacillus fermentum	Gastrointestinal_tract
Lactobacillus helveticus	Gastrointestinal_tract
Lactobacillus paracasei	Gastrointestinal_tract
Lactobacillus parafarraginis	Oral
Lactobacillus plantarum	Gastrointestinal_tract
Lactobacillus rhamnosus	Gastrointestinal_tract
Leuconostoc mesenteroides	Gastrointestinal_tract
Listeria innocua	Gastrointestinal_tract
Microbacterium	Oral
Myroides odoratimimus	Unknown
Neisseria	Oral
Neisseria lactamica	Airways

Paenibacillus	Gastrointestinal_tract
Paenisporosarcina	Gastrointestinal_tract
Pediococcus acidilactici	Gastrointestinal_tract
Peptoniphilus	Oral
Phascolarctobacterium succinatutens	Gastrointestinal_tract
Porphyromonas	Oral
Prevotella	Oral
Propionibacterium	Urogenital_tract
Propionibacterium acnes	Skin
Proteus penneri	Gastrointestinal_tract
Providencia rettgeri	Gastrointestinal_tract
Pseudomonas	Gastrointestinal_tract
Psychrobacter	Blood
Slackia	Oral
Staphylococcus	Gastrointestinal_tract
Staphylococcus aureus	Airways
Streptococcus	Airways
Streptococcus infantarius	Gastrointestinal_tract
Streptococcus infantis	Unknown
Streptococcus pseudopneumoniae	Airways
Streptococcus vestibularis	Oral
Streptomyces	Gastrointestinal_tract
Veillonella	Gastrointestinal_tract
Weissella paramesenteroides	Gastrointestinal_tract

Table 18: A list of all bacteria identified on the Experimental master list that were identified as part of the human microbiome (NIH Human Microbiome Project (NIHHMP)). Also listed on this table is the associated body site for the bacterium.

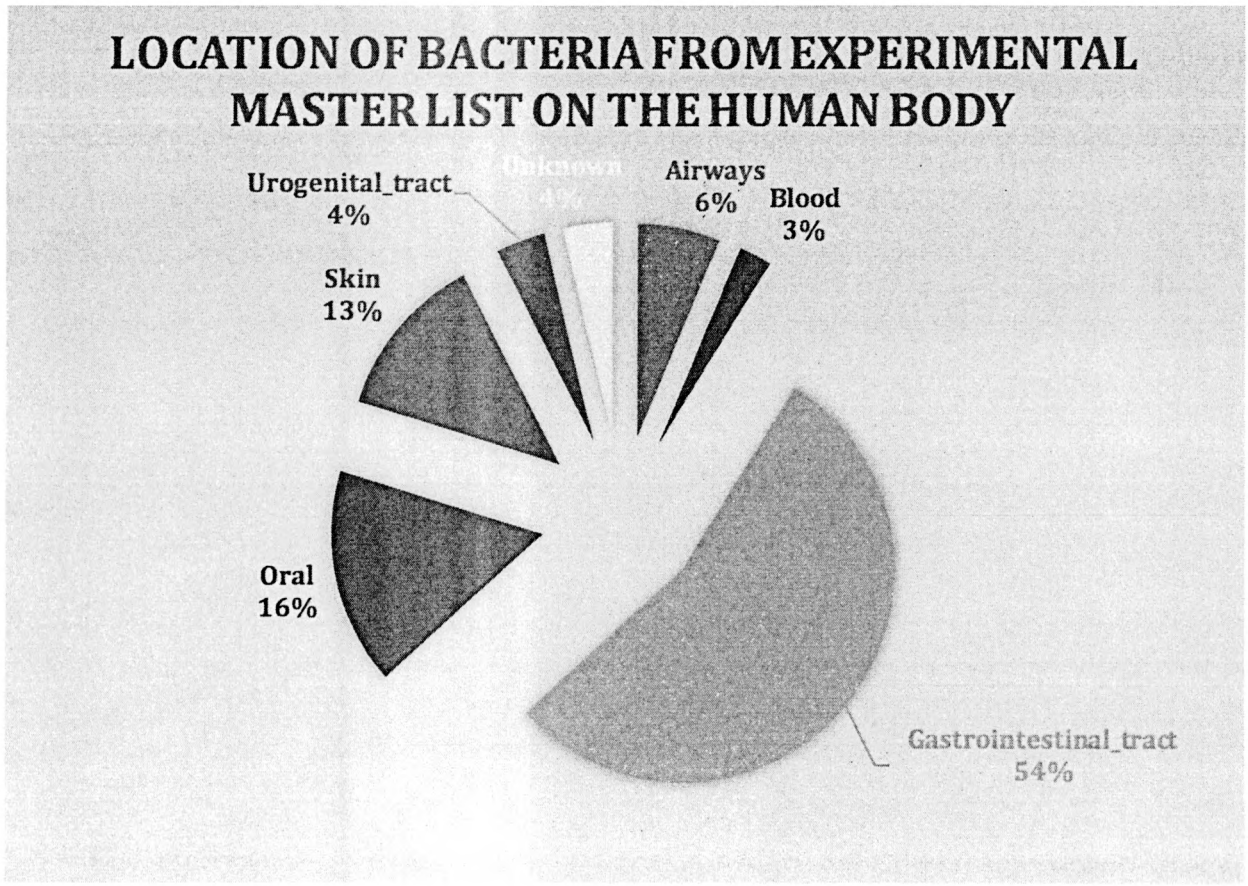


Figure 11: Of the 144 Experimental species that matched the HMP dataset, the proportions were greatest for the GI-tract (blue, 45%), oral (orange, 20%), urogenital tract (turquoise, 11%) and skin (purple, 9%). The entire circle represents 100% of the 144 species, and the sizes of each color represent the proportion of each type of bacteria.

Table 17 lists, in alphabetical order, the bacteria identified on the Control master list that were identified as part of the human microbiome (NIH Human Microbiome Project (NIHHMP)). Also listed on this table is the associated body site for the bacterium. This data is also graphically represented in Figure X following this table. Of the 67 Control species that matched the HMP dataset, the proportions were greatest for the GI-tract (40%), oral (25%), skin (13%) and urogenital tract (9%). The entire circle represents 100% of the 67 species, and the sizes of each color represent the proportion of each type of bacteria.

Bacteria from Control Master List	Human Body Sites
Acinetobacter	Skin
Acinetobacter baumannii	Skin
Acinetobacter johnsonii	Skin
Acinetobacter junii	Gastrointestinal_tract
Acinetobacter lwoffii	Skin
Acinetobacter radioresistens	Gastrointestinal_tract
Bacillus	Gastrointestinal_tract
Bacteroides	Gastrointestinal_tract
Bifidobacterium	Gastrointestinal_tract
Brevundimonas diminuta	Oral
Citrobacter	Gastrointestinal_tract
Clostridium	Gastrointestinal_tract
Clostridium perfringens	Gastrointestinal_tract
Corynebacterium	Gastrointestinal_tract
Corynebacterium ammoniagenes	Gastrointestinal_tract
Corynebacterium amycolatum	Skin
Corynebacterium genitalium	Urogenital_tract
Corynebacterium glucuronolyticum	Urogenital_tract
Corynebacterium pseudogenitalium	Urogenital_tract
Corynebacterium tuberculostearicum	Skin
Desulfovibrio	Gastrointestinal_tract
Enterobacter cancerogenus	Gastrointestinal_tract
Enterobacter hormaechei	Oral
Enterococcus	Gastrointestinal_tract

Enterococcus casseliflavus	Oral
Escherichia	Gastrointestinal_tract
Escherichia coli	Gastrointestinal_tract
Filifactor alocis	Oral
Fusobacterium	Oral
Fusobacterium necrophorum	Gastrointestinal_tract
Gemella haemolysans	Airways
Gemella sanguinis	Airways
Haemophilus	Oral
Haemophilus parainfluenzae	Skin
Klebsiella	Gastrointestinal_tract
Klebsiella oxytoca	Unknown
Lactobacillus	Gastrointestinal_tract
Lactobacillus plantarum	Gastrointestinal_tract
Megasphaera	Urogenital_tract
Neisseria	Oral
Neisseria lactamica	Airways
Paenibacillus	Gastrointestinal_tract
Peptoniphilus	Oral
Phascolarctobacterium succinatutens	Gastrointestinal_tract
Porphyromonas	Oral
Porphyromonas catoniae	Oral
Prevotella	Oral
Propionibacterium	Urogenital_tract
Propionibacterium acnes	Skin
Propionibacterium avidum	Oral

Pseudomonas	Gastrointestinal_tract
Psychrobacter	Blood
Ruminococcus	Gastrointestinal_tract
Selenomonas	Oral
Selenomonas infelix	Oral
Slackia	Oral
Staphylococcus	Gastrointestinal_tract
Staphylococcus aureus	Airways
Streptococcus	Airways
Streptococcus equinus	Urogenital_tract
Streptococcus infantarius	Gastrointestinal_tract
Streptococcus infantis	Unknown
Streptococcus oralis	Oral
Streptococcus pseudopneumoniae	Airways
Streptococcus vestibularis	Oral
Streptomyces	Gastrointestinal_tract
Veillonella	Gastrointestinal_tract

Table 17: A list of all bacteria identified on the Control master list that were identified as part of the human microbiome (NIH Human Microbiome Project (NIHHMP)). Also listed on this table is the associated body site for the bacterium.

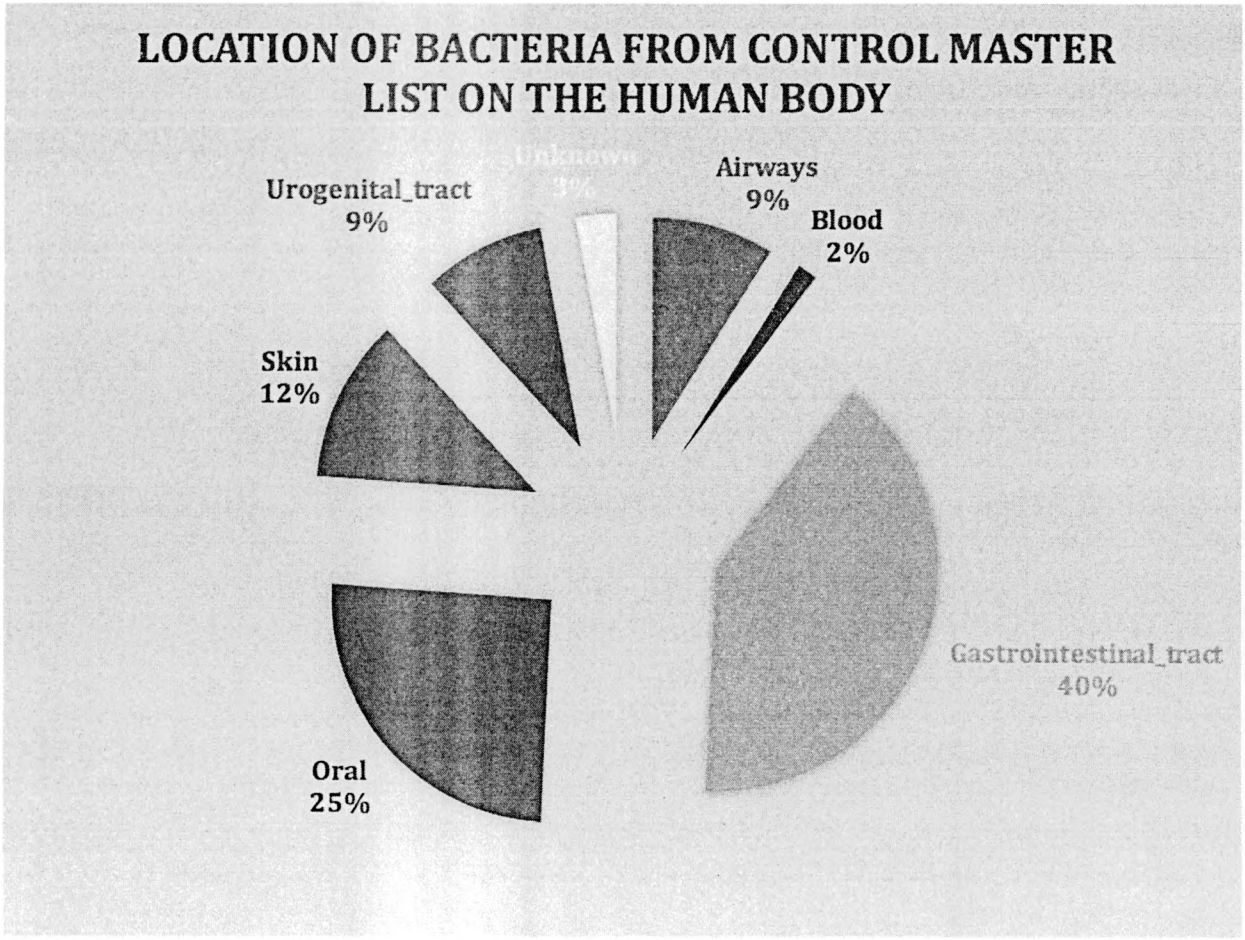


Figure 12: Of the 143 Control species that matched the HMP dataset, the proportions were greatest for the GI-tract (blue, 34%), oral (orange, 27%), urogenital tract (turquoise, 12%) and skin (purple, 10%). The entire circle represents 100% of the 143 species, and the sizes of each color represent the proportion of each type of bacteria.

Appendix E: List of Pathogenic Organisms

List of Pathogenic Bacteria						
Genus	**Species**	**Domain**	**Gram Stain**	**Shape**	**Oxygen Requirement**	**Info**
Achromobacter		Bacteria	G. Negative	Bacilli	Aerobic	Soil and water
Acinetobacter		Bacteria	G. Negative	Bacilli	Aerobic	Found in soil
	baumannii	Bacteria	G. Negative	Bacilli	Aerobic	Causes Infections
	lwoffii	Bacteria	G. Negative	Bacilli	Aerobic	Normal flora of people
Actinomadura		Bacteria	G. Positive	Bacilli	Aerobic	Madura foot
Actinomycetes		Bacteria	G. Positive	Bacilli	Anaerobic	Spore
	isaelii	Bacteria	G. Positive	Cocci	Anaerobic	Lesions
Aeromonas		Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Aquatic Environments
Afipia						
	felis	Bacteria	G. Negative	Bacilli	Aerobic	Cat Scratch Fever
Alcaligenes		Bacteria	G. Negative	Bacilli	Aerobic	Aquatic Environments
	xylosoxidans	Bacteria	G. Negative	Bacilli	Aerobic	Aquatic Environments
Aspergillus		Eukaryotic				Fungi
Bacillus						
	anthracis	Bacteria	G. Positive	Bacilli	Aerobic	Spore
	cereus	Bacteria	G. Positive	Bacilli	Aerobic	Spore
	subtilis	Bacteria	G. Positive	Bacilli	Aerobic	Spore
Bacteroides		Bacteria	G. Negative	Bacilli	Anaerobic	Lesions
	fragilis	Bacteria	G. Negative	Bacilli	Anaerobic	Common in Gut
Bartonella						
	bacilliformis	Bacteria	G. Negative	Bacilli	Aerobic	Oroya Fever
	henselae	Bacteria	G. Negative	Bacilli	Aerobic	Cat Scratch Fever
	pertusssis	Bacteria	G. Negative	Bacilli		Whooping cough
	quintana	Bacteria	G. Negative	Bacilli	Aerobic	Antigenic lesions
Bordetella						
	pertussis	Bacteria	G. Negative	Cocci	Aerobic	Respiratory Infection

Borrelia						
	afzelii	Bacteria	G. Negative	Spirochetes	Anaerobic	Lyme disease
	burgdorferi	Bacteria	G. Negative	Spirochetes	Anaerobic	Needs Vector
	duttoni	Bacteria	G. Negative	Spirochetes		Tick-borne Relapsing Fever
	garinii	Bacteria	G. Negative	Spirochetes	Anaerobic	Tick-borne Borreliosis
	recurrentis	Bacteria	G. Negative	Spirochetes		Relapsing Fever
Brevundimonas						
	diminuta	Bacteria	G. Negative	Bacilli	Aerobic	Non-Lactose-Fermenting
	vesicularis	Bacteria	G. Negative	Bacilli	Aerobic	
Brucella		Bacteria	G. Negative	Cocci	Aerobic	
	abortus	Bacteria	G. Negative	Bacilli	Aerobic	Naturally found in cattle
	canis	Bacteria	G. Negative	Bacilli	Aerobic	Naturally found in dogs
	melitensis	Bacteria	G. Negative	Bacilli	Aerobic	Goats or sheep
	suis	Bacteria	G. Negative	Bacilli	Aerobic	Swine-specific
Burkholderia						
	cepacia	Bacteria	G. Negative	Bacilli	Aerobic	Attacks the lungs
	mallei	Bacteria	G. Negative	Cocci	Aerobic	Domesticated animals
	pseudomallei	Bacteria	G. Negative	Bacilli	Aerobic	Cause pneumonia
Calymmatobacterium						
	granulomatis	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	
Campylobacter		Bacteria	G. Negative	Bacilli		
	jejuni	Bacteria	G. Negative	Bacilli	Microaerophilic	Food poisoning
Candida						
	albicans	Eukaryotic				Yeast
	krusei	Eukaryotic				Yeast
	parapsilosis	Eukaryotic				Yeast

Chlamydia						
	pneumoniae	Bacteria	G. Negative	Cocci		Intracellular parasites
	psittaci	Bacteria	NA	Cocci		Respiratory infections
	trachomatis	Bacteria	NA	Cocci		Obligate intracellular
Citrobacter		Bacteria	G. Negative	Bacilli	Facultative Anaerobic	
Clostridium						
	botulinum	Bacteria	G. Positive	Bacilli	Anaerobic	Spore
	difficile	Bacteria	G. Positive	Bacilli	Anaerobic	Spore
	perfringens	Bacteria	G. Positive	Bacilli	Anaerobic	Spore
	tetani	Bacteria	G. Positive	Bacilli	Obligate Anaerobic	Spore
Corynebacterium		Bacteria	G. Positive	Bacilli	Aerobic	
	diphtheriae	Bacteria	G. Positive	Bacilli	Aerobic	Disease diphtheria
	pseudotuberculosis	Bacteria	G. Positive	Bacilli	Facultative Anaerobic	Sheep and goat
	ulcerans	Bacteria	G. Positive	Bacilli		Dairy animals
Coxiella						
	burnetii	Bacteria	G. Negative	Bacilli	Aerobic	Q-fever
Cryptococcus						
	neoformans	Eukaryotic				Yeast
Enterobacter						
	aerogenes	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Human gastrointestinal flora
	cloacae	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Nosocomial pathogen
Enterococcus						
	faecalis	Bacteria	G. Positive	Cocci	Facultative Anaerobic	Intestines of mammals
	faecium	Bacteria	G. Positive	Cocci	Facultative Anaerobic	Urinary tract infection
	hirae	Bacteria	G. Positive	Cocci	Facultative Anaerobic	Non-motile cells
Epidermophyton		Eukaryotic	G. Negative	Bacilli	Facultative Anaerobic	Fungi
Escherichia						
	coli	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Intestines of humans
Francisella						
	tularensis	Bacteria	G. Negative	Bacilli	Aerobic	Plague-like illness

Gardnerella						
	vaginalis	Bacteria	G. Negative	Bacilli	Anaerobic	Sexually transmitted
Haemophilus						
	aegyptius	Bacteria	G. Negative	Bacilli		Conjunctivitis
	ducreyi	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Sexually transmitted
	influenzae	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Influenza
Helicobacter						
	pylori	Bacteria	G. Negative	Bacilli	Aerobic	Peptic ulcers
Histoplasma						
	capsulatum	Fungi				Eukaryotic
Klebsiella						
	granulomatis	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Sexually transmitted
	oxytoca	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Infectious spondylodiscitis
	pneumoniae	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Environmental organism
Leclercia						
	adecarboxylata	Bacteria	G. Negative	Bacilli	Aerobic	Human gut microbe
Legionella						
	pneumophila	Bacteria	G. Negative	Bacilli	Aerobic	Legionnaire's disease
Leptospira						
	interrogans	Bacteria	G. Negative	Spirochetes	Aerobic	Leptospirosis
	noguchii	Bacteria	G. Negative	Spirochetes		
	santarosai	Bacteria	G. Negative	Spirochetes	Aerobic	Zoonotic disease
	weilii	Bacteria	G. Negative	Spirochetes		
Leuconostoc						
	pseudomesenteroides	Bacteria	G. Positive	Cocci	Facultative Anaerobic	Produces lactic acid
Listeria						
	monocytogenes	Bacteria	G. Positive	Bacilli	Facultative Anaerobic	Listeriosis

Micrococcus						
	luteus	Bacteria	G. Positive	Cocci	Aerobic	Environmental organism
Microsporum		Eukaryotic				Fungi
Morganella		Bacteria	G. Negative	Bacilli	Aerobic	
Mycobacterium						
	avium	Bacteria	Acid Fast	Bacilli		Environmental organism
	chimaera	Bacteria	Acid Fast	Bacilli	Aerobic	
	leprae	Bacteria	Acid Fast	Bacilli	Aerobic	Smaller genome due to evolution
	tuberculosis	Bacteria	Acid Fast	Bacilli	Aerobic	Tuberculosis
	ulcerans	Bacteria	Acid Fast	Bacilli	Aerobic	Buruli and Bairnsdale ulcer
Mycoplasma						
	genitalium	Bacteria	NA	Cocci	Facultative Anaerobic	Sexually transmitted
	pneumoniae	Bacteria	NA	Cocci	Facultative Anaerobic	Respiratory Infection
Neisseria						
	gonorrhoeae	Bacteria	G. Negative	Cocci	Aerobic	Gonorrhea
	meningitidis	Bacteria	G. Negative	Cocci	Aerobic	Septicemia and meningitis
Nocardia		Bacteria	G. Positive	Bacilli	Aerobic	
Orientia						
	tsutsugamushi	Bacteria	G. Negative	Bacilli		Scrub typhus
Pantoea						
	agglomerans	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	
Pneumocystis						
	jiroveci	Eukaryotic				Fungi
Porphyromonas						
	gingivalis	Bacteria	G. Negative	Bacilli	Anaerobic	Periodontal disease
Prevotella		Bacteria	G. Negative	Bacilli	Obligate Anaerobic	
Propionibacterium						
	acnes	Bacteria	G. Positive	Bacilli	Anaerobic	Acne
Proteus						
	mirabilis	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Normal gut flora

	vulgaris	Bacteria	G. Negative	Bacilli	Aerobic	
Providencia						
	rettgeri	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Normal gut flora
	stuartii	Bacteria	G. Negative	Bacilli		Normal gut flora
Pseudomonas		Bacteria	G. Negative	Bacilli	Aerobic	
	aeruginosa	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Environmental organism
Ralstonia		Bacteria	G. Negative	Bacilli		
Rickettsia						
	akari	Bacteria	NA	Bacilli		Intracellular pathogens
	prowazekii	Bacteria	NA	Bacilli	Aerobic	Typhus
	rickettsii	Bacteria	NA	Bacilli		Rocky Mountain Spotted Fever
	tsutsugamushi	Bacteria	NA	Bacilli		Intracellular organism
	typhi	Bacteria	NA	Bacilli	Aerobic	Murine typhus
Rochalimaea						
	quintana	Bacteria	NA	Bacilli		Louse-born trench fever
	gilardii	Bacteria	G. Negative	Bacilli	Aerobic	
Salmonella		Bacteria	G. Negative	Bacilli	Facultative Anaerobic	
	enteritidis	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Typhoid fever
	paratyphi	Bacteria	G. Negative	Bacilli	Aerobic	
	typhi	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	
	typhimurium	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	
Serratia						
	marcescens	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Environmental organism
Shigella						
	boydii	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Uncommon except in India
	dysenteriae	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Shigella-related deaths
	flexneri	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Endemic infections
	sonnei	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Mild diarrhea and dehydration
Sphingomonas		Bacteria	G. Negative	Bacilli	Aerobic	

Staphylococcus						
	aureus	Bacteria	G. Positive	Cocci	Facultative Anaerobic	Nosocomial infections
	capitis	Bacteria	G. Positive	Cocci	Aerobic	Bloodstream of a premature infant
	epidermidis	Bacteria	G. Positive	Cocci	Facultative Anaerobic	Normal human skin flora
	haemolyticus	Bacteria	G. Positive	Cocci	Facultative Anaerobic	Normal human skin flora
	hominis	Bacteria	G. Positive	Cocci	Facultative Anaerobic	Normal human skin flora
	lugdunensis	Bacteria	G. Positive	Cocci	Aerobic	Skin and soft tissue infections
	saprophyticus	Bacteria	G. Positive	Cocci	Facultative Anaerobic	Normal human skin flora
Stenotrophomonas						
	maltophilia	Bacteria	G. Negative	Bacilli	Aerobic	Breathing tubes
Streptobacillus						
	moniliformis	Bacteria	G. Negative	Bacilli	Microaerophilic	Rat bite fever
Streptococcus		Bacteria	G. Positive	Cocci	Anaerobic	
	agalactiae	Bacteria	G. Positive	Cocci	Facultative Anaerobic	Meningitis and sepsis
	mutans	Bacteria	G. Positive	Cocci	Facultative Anaerobic	Tooth decay
	pneumoniae	Bacteria	G. Positive	Cocci		Normal human nasopharyngeal flora
	pyogenes	Bacteria	G. Positive	Cocci		Normal human nasopharyngeal flora
Streptomyces		Bacteria	G. Positive	Bacilli		Spore
Treponema						
	pallidum	Bacteria	G. Negative	Spirochetes	Anaerobic	Syphilis
Trichophyton		Eukaryotic				Fungi
Trichosporon		Eukaryotic				Fungi
Ureaplasma						
	urealyticum	Bacteria	NA			Normal human urogenital tract flora
Vibrio						
	cholerae	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Colonizes mucosa of small intestines
	parahaemolyticus	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Food poisoning

Yersinia						
	enterocolitica	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Food and waterborne pathogen
	pestis	Bacteria	G. Negative	Bacilli	Facultative Anaerobic	Bubonic plague
	pseudotuberculosis	Bacteria	G. Negative	Bacilli	Anaerobic	Lesions in the lung